APPLYING BIODIVERSITY AND ECOSYSTEM FUNCTIONING THEORY TO URBAN GRASSLAND MANAGEMENT

A Thesis

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ABSTRACT

Urban grasslands are rapidly expanding in the United States and around the world. Such landscapes are dominated by turfgrasses and are kept as aesthetic or recreational groundcovers. These systems are less diverse than the natural or agricultural systems they replace, leading to potential losses in ecosystem functioning. Research in prairie grassland ecosystems suggests increasing aboveground diversity may enhance multiple ecosystem services. Biodiversity-ecosystem functioning (BEF) research has linked species richness to enhanced productivity, resilience, invader resistance, and overall multi-functionality. Therefore, there is potential to improve the ecosystem services of urban landscapes if biodiversity is explicitly considered in their design and maintenance.

An urban grassland diversity gradient was studied in greenhouse mesocosms to determine biodiversity effects on productivity and nitrate leaching. Twelve turfgrass components were randomly assigned to four diversity level treatments: monoculture or 3, 6, or 12-part polycultures. Productivity was examined over 17 weeks by measuring total aboveground and belowground yield. Nitrate leaching was assessed by quantifying nitrate-nitrogen (NO_3^--N) losses recovered from lysimeters.

Plant diversity stimulated total and standing productivity between monoculture and 3-component polyculture treatments. Positive trends were observed for 6- and 12-component polycultures compared to monocultures for standing aboveground harvest and total biomass. No differences were observed in belowground productivity. Experimental observations presented here match literature trends, generally showing positive productivity responses to aboveground diversity. Highly productive species effects are driving some of the observed productivity trends. Yet, selection of diverse turf assemblages does increase the likelihood of including highly

productive species, thus aboveground richness is still a valuable means for enhancing urban grassland productivity.

Aboveground diversity reduced NO_3^--N concentrations in leachate. When a nitrogen (N) fixing legume was included in turf assemblages, NO_3^- leaching increased, but not significantly more than other assemblages within the same diversity level treatment. Nitrate losses decreased up to four fold in the highest diversity polyculture compared to the monoculture legume treatment. Nitrate leaching trends observed in experimental mesocosms concur with published results in the literature, supporting the conclusion that polycultures retain N more effectively than monocultures.

Polycultures also enriched for greater diversity of soil microbiota. The gradient of plant diversity did not show an increasing level of bacterial or fungal diversity. Instead, it mattered whether there was a single plant species versus multiple plant species. A similar pattern held when the structure of the microbial community showed greater variability in monoculture, but similar profiles when multiple plant species were present.

This study agrees with previous BEF research, showing biodiversity effects enhance multiple ecosystem services. Future studies should focus on ecosystem processes relevant to urban grasslands such as carbon storage, N retention, and resistance to biological invasions. Furthermore, studies should assess the strengths of biodiversity effects *in situ* and determine the scales at which diversity enhances multiple ecosystem processes.

BIOGRAPHICAL SKETCH

Grant L. Thompson was born in Des Moines, Iowa to Jerome and Gretchen Thompson. In 2002, he graduated from Hoover High School and Central Academy. He attended Iowa State University in Ames, Iowa as a third generation Cyclone following both his parents and grand father. In 2007 he earned dual bachelors degrees in Horticulture and Landscape Architecture, cum laude, with honors and distinction. His Horticulture advisor Prof. Ann Marie VanDerZanden, a Cornell University graduate, nominated him to be student marshal for the College of Agriculture and Life Sciences at the graduation ceremonies.

Upon graduation he entered the work force at Genus Landscape Architects in Des Moines. While at Genus, Grant was promoted to project landscape architect after earning his professional license in 2011. During his career he served on the Executive Committee of the Iowa Chapter of the American Society of Landscape Architects. It was at Genus, Grant found his passion for assessing the impacts of urban landscapes and sought ways to measurably improve sustainable design and best management practices.

Grant entered Cornell University in the fall of 2012 in the Field of Horticulture through the MS/PhD program.

DEDICATION

I dedicate this to my parents. Their belief in me has never wavered. Thank you for the sacrifices you have made to give me the experiences that have shaped who I am today.

I dedicate this to Thressa, my wife. Your belief in my dream brought us across the country, away from home, work, and friends. Your faith in my pursuits cannot be repaid. To you, I dedicate this.

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CHAPTER ONE

APPLYING ECOLOGICAL THEORY TO THE DESIGN OF URBAN GRASSLANDS

Introduction

Urban grasslands, such as turfgrass ecosystems, have expanded rapidly in the United States in recent decades and will continue to become a dominant vegetation cover in developed landscapes. Within a 15 year period (1982-1997) urbanization expanded in the U.S. by 50% (Fulton *et al.* 2001). Although turfgrass lawns comprise only a portion of developed landscapes, collectively they cover 2% of the total terrestrial land area of the U.S. In fact, American lawns cover an area three times larger than any irrigated crop (Milesi *et al.* 2005). The continuing expansion of developed lands suggests that expanded urban grasslands will be a consequence of urbanization.

In 2010, the U.S. Census Bureau found that over 80% of the nation lives in urban areas. The trend toward urbanization has increased by approximately 1.8% since 2000 (Bureau 2011). Remote sensing analysis of recently subdivided suburban parcels suggest between 25% and 90% of the landscape was pervious, much of which is likely to include lawns (Cappiella & Brown 2001). The dominance of turfgrass in developed landscapes is evidenced by a study conducted in a representative Ohio urbanized landscape showing that 23% of the land area was covered with turfgrass lawns (Robbins & Birkenholtz 2003). Similarly, an extensive study of the Chesapeake Bay watershed showed a 61% (3,186 km^2) increase in urbanized land from 1990 through 2000, with much of the vegetated landscape likely dominated by lawns (Jantz *et al.* 2005).

Replacing other ecosystems with urban grasslands across the U.S. could lead to a decline of ecosystem services. Expanding turfgrass coverage can increase the potential for non-point source pollution from fertilizer mismanagement, overall reduced biodiversity, and increased greenhouse gas emissions from associated maintenance practices (Barth 1995; Bormann *et al.* 2001; Guillard & Kopp 2004; Townsend-Small & Czimczik 2010; Raciti *et al.* 2011a). Although turfgrass landscapes pose several ecological concerns, multiple studies have indicated the potential to enhance nitrogen (N) retention and carbon (C) storage in soils under turfgrasses (Qian & Follett 2002; Bandaranayake *et al.* 2003; Golubiewski 2006; Raciti *et al.* 2008; Groffman *et al.* 2009; Townsend-Small & Czimczik 2010). While lawns offer more beneficial ecosystem services than paved surfaces, there is great potential to enhance ecosystem performance by reimagining the American lawn.

Traditional strategies for improving turfgrass ecosystems have focused on reducing inputs through improved breeding programs or by utilizing native turf species (Simmons *et al.* 2011). While such approaches will continue to be important, additional benefits of urban grasslands may be possible if lessons from ecological theory are applied to turf assemblage selection and design. Here, we discuss turfgrass systems in the context of urban grasslands, identify key ecosystem processes altered by lawns, and evaluate the potential to integrate biodiversity into designed landscapes. Specifically, we summarize the fundamental principles of BEF research relevant to C storage, N retention, and resistance to weed invasion in grasslands and prairies. Greater understanding of the mechanisms underlying biodiversity enhancement of ecosystem functions will facilitate the incorporation of biodiversity at many levels in the urbanized landscape.

Defining urban grasslands

Urban grasslands differ from native grasslands or forage-type grasses in species composition and management techniques. Urban grasslands are considered to be landscapes consisting of turf-type grasses, generally characterized as semi-regularly mown to a height of 10 cm (about 4 inches) or less, may be irrigated, may be fertilized, and are maintained as a ground cover for light traffic, sports surfaces, or aesthetic reasons (Groffman *et al.* 2009). A range of management practices and intensities are encompassed by this broad definition of urban grasslands. In this manuscript *turf* landscapes include the most highly managed urban grasslands, consisting of only select graminaceous species, intensively mown, frequently fertilized and irrigated, and treated with pesticides. Turf landscapes are typically synonymous with sports fields, golf courses, and the most intensively maintained residential or civic green spaces. *Lawns* are less tightly controlled and may include desirable broad-leaf plants, have a moderate tolerance for weeds, are mown infrequently, receive little if any additional fertilization or irrigation, and are subject to reduced pesticide application. Lawn landscapes are typical of low-use-intensity municipal parks, corporate or college campuses, and a majority of residential properties.

The typical American urban grassland is dominated by one to three turfgrass species. In cooler climates, Kentucky bluegrass (*Poa pratensis*) is the most common species because it provides good traffic tolerance in a recreational or sport setting. It is also common to find *P. pratensis* growing with perennial ryegrass (*Lolium perenne*). In more recent times, tall and fine fescues have become common in residential and civic lawns. In warmer climates, Bermuda grass (*Cynondon dactylon*), centipede grass (*Eremochloa ophiuroides*), and St. Augustine grass (*Stenotaphrum secundatum*) are the most common turfgrasses.

Of the over 7,500 species within the Poaceae family, only a few dozen will tolerate the cultural maintenance practices necessary in urban grasslands. Thus, urban grasslands are low diversity, when compared to native ecosystems (Turgeon 2005). Growing conditions are used to categorize turfgrass species, rather than the subfamily delineations of Pooid, Chloridoid, and Panicoid (Table 1.1).

Table 1.1 – Turfgrass *Poaceae* subfamily characteristics, adapted from Turgeon (2005).

	Poaceae Subfamilies		
Characteristics	Pooid	Chlordoid	Panicoid
Season class	Cool Season	Warm Season	Warm Season
Climatic zones	Cool temperate, subarctic, subtropical	Tropical, subtropical, warm temperate	Tropical, subtropical
Photosynthetic pathway	C_3 carbon fixation	C_4 carbon fixation	C_4 carbon fixation
Turfgrass tribal groups	*Festuceae, Aveneae, Triticeae*	*Chlorideae*	*Paniceae, Andropogoneae*

Thinking of urban grasslands in terms of ecological services

In order to improve the ecological services of urban grasslands, key ecosystem functions and their drivers must be considered. Biogeochemical cycling, especially carbon and nitrogen, are widely impacted by the cultivation of urban grasslands (Qian & Follett 2002; Milesi *et al.* 2005; Pouyat *et al.* 2006; Pouyat *et al.* 2009; Raciti *et al.* 2011b). Additionally, the decrease of plant diversity reduces natural resistance to invasion, necessitating increased management efforts to suppress weeds (Hector *et al.* 2002). Ecological theory derived from empirical studies in native prairie ecosystems can inform the establishment of urban grasslands, which actively support desired ecosystem functions.

Ecosystem functions include all processes carried out by an ecosystem, for example nutrient uptake and conversion, as well as energy flow between trophic levels; particular processes of value to human society are termed *ecosystem services*. The 2005 Millennium

Ecosystem Assessment classifies services provided by an ecosystem as supporting, provisioning, regulating, or culturally relevant benefits. Ecosystem services – and thereby ecosystem functions – can be radically different in natural landscapes and those impacted by human development. Loss of biodiversity can reduce the functioning of ecosystems including negatively impacting productivity, stability, resistance to invasion, and nutrient dynamics (Assessment 2005). Functional reduction is a concern for a wide range of ecosystems in communities around the world – including urban grasslands.

Substantial theoretical and experimental work has probed the functional outcomes of biodiversity in ecosystems. Early explorations defined diversity as species richness, then as functional groups, and more recently in terms of phylogenetic distance. However, many studies continue to use species richness as the primary diversity metric since richness is easy to manipulate and measure. Experimental and observational studies of native grasslands, drylands, forests, and aquatic ecosystems represent the majority of biodiversity and ecosystem functioning (BEF) research to date (Balvanera *et al.* 2006; Worm *et al.* 2006; Cadotte *et al.* 2008; Cardinale *et al.* 2011; Maestre *et al.* 2012). Yet, relatively little BEF research has been conducted within urban grassland systems. In a study of native vs. non-native turfgrasses, Simmons *et al.* (2011) considered diversity, but only among native turf treatments, hence biodiversity effects were not fully explored within urban grasslands. The next section provides an overview of the conceptual origins, current thinking, and mechanisms by which biodiversity have been studied in relation to ecosystem functions. Incorporating biodiversity in urban grasslands has the potential to partially mitigate detrimental environmental impacts associated with the conversion of native ecosystems to urbanized lands.

Underlying principles of Biodiversity-Ecosystem Function research

Decades of scientific debate have attempted to conceptualize, experientially determine, and model the role biodiversity plays in determining ecosystem functioning. The dramatic surge in BEF research resulted from an early international conference on biodiversity held in Bayreuth, Germany in 1991 (Schulze & Mooney 1994). The meeting catalyzed much research resulting in the second conference in Paris, France in 2000 to summarize a decade of data (Loreau 2002). Subsequent research has attempted to reconcile theoretical and experimental findings, uncover biodiversity effects in various ecosystems, refine the spatial and biological scales at which diversity is measured, and identify mechanisms driving biodiversity effects. A more complete review of theoretical foundations, historic discourse, and current directions in BEF research will not be covered here, see (Hooper *et al.* 2005; Cardinale *et al.* 2006; Cadotte *et al.* 2008; Hooper *et al.* 2012; Loreau & Mazancourt 2013).

Early hypotheses suggested *linear*, *redundant*, *keystone*, or *rivet* response models to characterize the relationship between an increasing biodiversity gradient and any given ecosystem process, see Figure 1.1 (Vitousek & Hooper 1993; Naeem *et al.* 2002). While the field has moved beyond these simplified models, they are still archetypes referenced during analysis of contemporary findings (Naeem *et al.* 2002). Generally, each model depicts the effect on a specific ecosystem process for each subsequent addition or subtraction of a species. A *linear* response indicates that species possess unique affects on a given process, with similar magnitudes, thus there is a direct relationship between the total number of species and the process of interest. *Redundant* models assume species have similar effects on ecosystem processes therefore increasing species richness produces a diminishing response, causing an asymptotic function (Lawton & Brown 1993). *Keystone* and *rivet* response models are related

and suggest a tipping point where if a critical species is lost (*keystone*) or diversity threshold is crossed (*rivet*), there will be a sharp decline in the ecosystem process of interest (Ehrlich 1981; Mills *et al.* 1993). To a great degree, early empirical work focused on a readily quantifiable ecosystem process, such as aboveground plant biomass production.

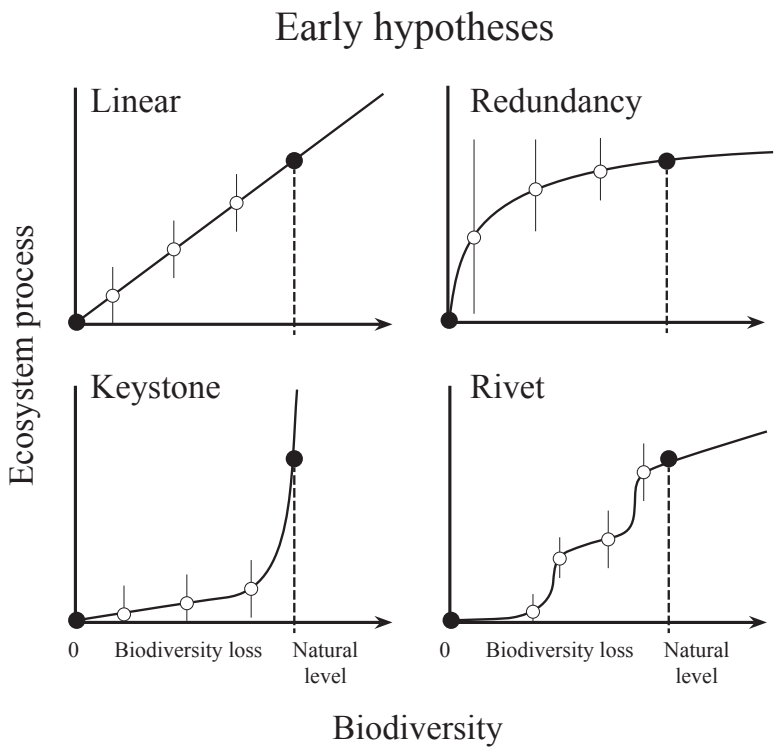

Figure 1.1 – Early hypotheses in BEF research postulated *linear*, *redundancy*, *keystone*, and *rivet* responses in ecosystem processes to declining biodiversity. Contemporary BEF research refers to these archetypal responses, but now considers functional responses to biodiversity loss to be more nuanced. This figure is reproduced and modified from (Naeem *et al.* 2002).

To understand why diversity would produce different response models and under what conditions, researchers focused on plant traits that might influence productivity. Initial BEF theory assumed that plant species possess different inherent traits with regards to nutrient acquisition, growth, and other life history aspects. These differences should result in a single species occupying a distinct role (niche) within an ecosystem. As species diversity increases, the

theory suggests a greater degree of *niche partitioning* occurs allowing multiple species to coexist and more efficiently utilize a finite set of environmental resources (for example nutrients, water, space, light, etc.), see Figure 1.2 (Tilman *et al.* 1997a; Tilman 1999). A specific extension of the niche partitioning theory is *species complementarity*, which occurs when i) resources utilized by multiple species occur in complementary ways in space or through time or ii) when interspecific interactions between two coexisting species enable more efficient utilization of resources (Cardinale *et al.* 2007). An alternative explanation for increased productivity associated with high-diversity communities is the *sampling effect*. This framework poses that polycultures possess a greater likelihood of including highly-productive species compared to monocultures (Aarssen 1997). Parsing out the mechanisms driving biodiversity effects is frequently difficult, may vary from ecosystem to ecosystem, and are interpreted differently among researchers (Naeem *et al.* 2002). Regardless of the mechanism, a majority of studies find diversity increases the average productivity of an ecosystem (Cardinale *et al.* 2013; Pasari *et al.* 2013).

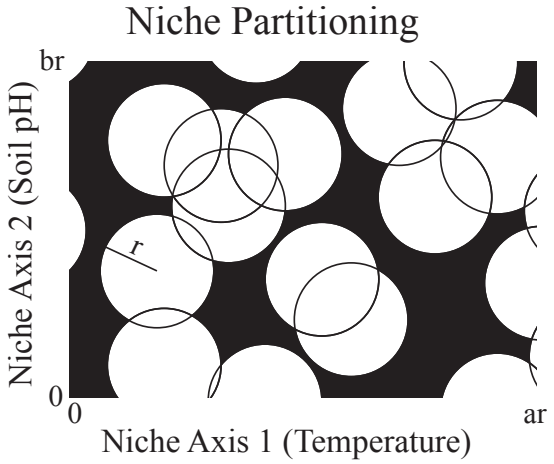

Figure 1.2 – Niche partitioning explains how a single species captures environmental resources within an ecosystem. However, no species is optimally suited to fully exploit the entire range of an ecosystem, for example at all temperature and soil pH combinations. As biodiversity increases, the entire niche space of an ecosystem is more fully utilized. This figure is reproduced from (Tilman *et al.* 1997b).

Biological diversity creates a stabilizing effect, moderating fluctuations in ecosystem functioning due to biotic and abiotic stresses (Hooper *et al.* 2005; Tilman *et al.* 2006). Ecosystem *stability* occurs when a community can sustain a process at an average level under fluctuating

environmental conditions, though species-level population fluctuations may occur. Long-term studies of a native prairie diversity gradient conducted at Cedar Creek, MN exhibit some of the strongest evidence coupling diversity and stability (Tilman *et al.* 2006). Tilman and colleagues found greater ecosystem stability with regards to biomass in 70% of high-diversity plots compared to monocultures. However, individual species stability within a polyculture was inversely related to species richness (Tilman *et al.* 2006). The authors concluded this was an example of the biodiversity *portfolio effect*, a generalized form of ecosystem stability. It should be noted that productivity and stability are not inherently linked and may vary independently in diverse ecosystems (Cardinale *et al.* 2013).

The conceptual framework describing trade-offs in polycultures, explained below, is borrowed from stock trading analogies in economics, and thus it is called the *portfolio effect* (Figge 2004). For example, under the most favorable conditions, a monoculture could be highly functional, but under stressed conditions (such as drought, disease, pests, etc.) the community could decline, losing functionality. In a multiple species scenario, one species may thrive while another declines under a given set of stresses. However, if environmental conditions change, the relative success of each species may be altered. Thus, a diversified community (or stock portfolio) can more adequately buffer changing stresses, but cannot capitalize on potentially large gains should conditions favor one species as compared to a monoculture of the favored species.

Current BEF research tends to focus on the *multi-functionality* of diverse ecosystems and alternative ways to consider diversity. Reassessments of long-term grassland studies have found plot scale species richness (α-diversity) and landscape scale heterogeneity (β-diversity) is necessary for ecosystems to simultaneously enhance multiple ecosystem functions (Zavaleta *et*

al. 2010; Pasari *et al.* 2013). In addition to assessing diversity within and between sites, a shifting focus of BEF research is emphasizing the predictive power of functional diversity (FD) and phylogenetic diversity (PD) (Cadotte *et al.* 2008; Cadotte *et al.* 2009; Flynn *et al.* 2011). Plant species vary with regards to functional traits, such as photosynthetic pathway (C_3, C_4, and CAM) and ability to fix nitrogen (legumes). Therefore combinations of FD have been suggested to be more relevant than species richness as a predictor of ecosystem function. Similarly, distantly related species (larger PD) are thought to be less similar in their functional traits, ecological niches, and life history strategies, and therefore more complementary than two closely related species (Cadotte *et al.* 2008; Cadotte *et al.* 2009).

The value of biodiversity, measured in species, traits, phylogeny, and at various scales, is strongly supported by the body of BEF research. While the mechanisms, strengths, and conditions under which biodiversity affects ecosystem processes remain unresolved, the consensus of the field is that diversity matters. Preserving or enhancing biodiversity within managed ecosystems is critical to sustaining the services they provide to human populations. To date, the design of urban grasslands has not included BEF theory to increase the multi-functionality of these landscapes.

Ecosystem services provided by urban grasslands

Urban grasslands have impacts on multiple ecosystem services in developed landscapes, especially when considered in the context of their rapid expansion. With regards to BEF theory, we will examine three services altered by grasslands: C storage, N retention, and (plant) invasion resistance. The following examples will discuss how these services are impacted in grasslands,

applicable BEF theory, and how increasing diversity in urban grasslands might enhance ecosystem functioning of lawn landscapes.

Carbon storage

Since land use conversion to urban conditions are likely to be more persistent (Pouyat *et al.* 2002), potential C storage gains from urban grasslands is a low-risk payoff worth considering. Urbanization causes a shift in terrestrial C cycling as land is converted from forest or agricultural uses (Pataki *et al.* 2006). Carbon cycling in urban grasslands is also substantially different than in natural environments due to human management practices, such as irrigation, fertilization, and mowing (Zhang *et al.* 2013b). Carbon cycling in urban environments remains poorly quantified, though research in urban grasslands suggests there is great potential for C sequestration, especially in belowground pools (Pouyat *et al.* 2002; Zhang *et al.* 2013b). Soil C stocks in turfgrass environments are typically greater than agricultural systems and can approach levels observed in native forests (Raciti *et al.* 2011b). However, C emissions from maintenance practices (e.g. fossil fuel consumed by mowing, embodied energy in fertilizers, and energy for irrigation) may decrease or completely offset belowground urban grassland C storage (Townsend-Small & Czimczik 2010; Zhang *et al.* 2013b). Carbon-poor agricultural soils have greater potential to accumulate soil C stocks than forest soils beginning with moderate soil C levels when converted to urban uses (Pataki *et al.* 2006).

Carbon storage may be significant in turfgrass systems where disturbance is minimized, sufficient water (natural or irrigated) exists, and fertilization allows soil organic matter (SOM) to increase. In one study low annual disturbance, increased inputs, and more pervious surfaces caused an increase of 44% and 38% in soil C densities

between low density residential and institutional land uses, respectively, compared to commercial developments (Pouyat *et al.* 2002). As SOM is approximately 57% C, increasing SOM is necessary to sequester C (Follett *et al.* 1987). Soils under a golf course in New York City were found to have the highest soil organic carbon (SOC) density (28.5 kg m^{-2}) of samples taken in six U.S. cities and Moscow, Russia (Pouyat *et al.* 2002). SOC was found to increase at the rate of 0.1 kg m^{-2} yr^{-1} over a 25 to 30 year period in golf course greens and fairways (Qian & Follett 2002) which is similar to SOC accumulation rates observed in ornamental lawns (0.14 kg C m^{-2} yr^{-1}) (Townsend-Small & Czimczik 2010). Modeling efforts predict similar accumulation rates of 2.3 to 3.2 kg m^{-2} SOC in the top 20 cm over 30 years for the conversion of native grasslands to golf turf in Colorado (Bandaranayake *et al.* 2003). In each of these instances, C emissions for urban grassland establishment and maintenance are not accounted for, so the net effect on global greenhouse gas (GHG) concentrations remains unknown.

Diversifying urban grasslands has the potential to increase the C sequestration of urban landscapes. There is overwhelming evidence indicating that plant species richness increases the efficiency of an ecosystem to utilize and convert assimilated resources into plant biomass (Hooper *et al.* 2005; Cardinale *et al.* 2007; Cadotte *et al.* 2008; Cardinale *et al.* 2011). These analyses summarize and parse over 20 years of plot, greenhouse, and mesocosm studies in which species diversity was directly manipulated in to assess how ecosystem functions, including productivity, were affected. Yet aboveground biomass accumulation does not necessarily account for C sequestration, as greater than 70% of terrestrial C is retained in soil stocks (Catovsky *et al.* 2002); this is of particular importance in turfgrass systems where mowing regularly removes aboveground biomass.

Enhancing the productivity of grasslands by increasing assemblage diversity could increase belowground productivity and rhizodeposition of C compounds, potentially contributing to an higher SOC accumulation rates.

As suggested by the *portfolio effect* and *stability* (discussed in the principles of BEF section), evidence shows that polycultures are frequently more productive than monocultures, but exceptions may exist. Cardinale *et al.* (2007) analyzed 44 experiments manipulating diversity and found that 79% of polycultures were more productive than average monoculture treatments. However, in 88% of these cases, the most productive individual species included in the polyculture, when grown in monoculture, accumulated more biomass than the polyculture (Cardinale *et al.* 2007). Upon further analyses of diversity experiments Cardinale *et al.* (2011) found the highest diversity polycultures only accumulated 87% of the biomass of the highest yielding monoculture. Thus, a diverse community would be expected to *on average* be more functional than an *average* monoculture but less functional than the *best-suited* monoculture. However, in application, rarely a) do we know the best monoculture, b) is the best monoculture ideal for all conditions of a specific ecosystem, and c) is the best monoculture optimized for ecosystem multi-functionality.

Given these constraints and the desire to increase C storage, diverse urban grasslands should be utilized where environmental conditions (spatially and temporally) are variable, multiple functions are desired from the lawn, and the ideal turfgrass selection is unknown. Although urban C storage represents a small portion of total soil C storage, diversifying urban grasslands is a low-risk opportunity to increase soil C in developed landscapes.

Nitrogen leaching

Nitrogen cycling is complex, highly variable, and subject to extensive human influence in urban grasslands. A study conducted by the National Gardening Association in 2000 found that 89.3% of American homes used fertilizers on their lawns or gardens. correlated with this are users of lawn chemicals and fertilizers that tend to be more highly educated, affluent, and self-identify as environmentally knowledgeable (Robbins *et al.* 2001). A study of the Minneapolis, MN metropolitan area found an excess of N inputs, predominantly from fertilizers, 51% greater than annual N demand for residential landscapes (Fissore *et al.* 2012). Increased application of N fertilizers on urban grasslands has resulted in higher N exports compared to forest ecosystems (Groffman *et al.* 2009). Turf fertilizers, especially synthetic formulations, are implicated as a non-point source of water pollution resulting in eutrophication of urban watersheds and contributing to algal blooms in coastal waters and estuaries (Petrovic 1990; Barth 1995; Easton & Petrovic 2004). Furthermore, turf fertilization can have significant contribution to climate change from nitrous oxide (N_2O) emissions, since N_2O has nearly 300 times the global warming potential of CO_2 on a per molecule basis (Raciti *et al.* 2011a; Zhang *et al.* 2013b).

Surprisingly, urban grasslands have displayed unexpectedly high capacities for N retention. In a review of multiple studies, Petrovic (1990) found greater than 90% of N was retained in turfgrass systems and this prevented N from leaching into groundwater. Similarly, several studies found unfertilized or minimally fertilized turfgrass could retain as much as 95% of N on an annual basis (Gold *et al.* 1990; Guillard & Kopp 2004). In long-term study plots nitrate (NO_3^-) leachate concentrations from urban grasslands showed N retention from applied fertilizer was near 100% in dry years and >60% in wet

years. Nitrogen removal through denitrification occurs at variable but low rates in urban grasslands (Raciti *et al.* 2011a). Multiple studies suggest the worst-case scenarios for high N leaching from urban grasslands include recently established lawns, turfgrass planted on heavily compacted soils or subsoils, over watering, and over fertilization (Morton *et al.* 1988; Easton & Petrovic 2004; Guillard & Kopp 2004; Cheng *et al.* 2013).

Application of BEF theory to urban grasslands would suggest increased turf-species diversity within lawns would aid in N retention because of more complete resource utilization (Loreau 1998). Indeed, Tilman *et al.* (1996) found soil NO_3^- to be a negative saturating function of species richness within and below the rooting zone of native grassland field mesocosms. Greater than a 50% decrease in soil NO_3^- was observed in their diverse polycultures that included six or more species. However, soil NO_3^- content only infers N leaching potential. Another study found that increasing both species richness and functional group richness caused a reduction in measured NO_3^- leaching, but leaching differences were only observed when an N fixing legume was included in the plot (Scherer-Lorenzen *et al.* 2003). Where legumes were not present, virtually no NO_3^- leached from plots suggesting that regardless of the number of species present non-N fixers will efficiently utilize available soil NO_3^- (Scherer-Lorenzen *et al.* 2003). In an effort to study biodiversity effects under future elevated atmospheric CO_2 concentrations, one study utilized free air C enrichment in combination with N fertilized and unfertilized native grassland plots (Mueller *et al.* 2013). Results from 13 years of the BioCON experiment show soil NO_3^- decreased both in response to species richness and functional group regardless of N fertilization. Fine root biomass leading to N uptake was suggested as the primary driver of observed diversity differences in soil NO_3^- (Mueller *et al.* 2013).

This study indicates that biodiversity effects in grasslands will continue to occur under elevated atmospheric CO_2 concentrations.

Given the strong evidence indicating that native grassland polycultures reduce soil NO_3^- and decrease N leaching potential, it seems logical to diversify urban grasslands. Complementarity effects in fine root biomass, N uptake, and N fixation have been used to explain the observed results from BEF research in native grasslands. While legumes are known to greatly increase the amount of soil NO_3^- and thus potentially increase N leaching, evidence suggests that this is reduced in diverse grassland polycultures (Scherer-Lorenzen *et al.* 2003; Mueller *et al.* 2013). Clover, *Trifolium spp.*, is a common weed in turf systems under moist, nutrient poor conditions (Turgeon 2005) and has been improved through breeding programs to achieve a dwarf form for use as a green alternative to N fertilizers (Wagner *et al.* 2010). In either case, unintended or deliberate N-fixation may decrease ground water quality unless diverse urban grasslands are present to utilize additional fixed-N.

Invasion resistance

Weed invasion is a common issue in urban grasslands, requiring significant time and expense to manage. Historically, pesticides were used to control disease, weed, and pest problems in turf; however as environmental and human health concerns have increased, many landscape managers are minimizing pesticide use. Nationally, consumer attitudes exhibit a shift away from turfgrass lawns managed through intensive pesticide and fertilizer application. Pesticide use has dropped from 139 to 127 million pounds annually between 1988 to 2007, while the cost has risen from $1.27 to $2.66 billion

dollars during the same period (EPA 2011). States such as California and New York, urged by similar legislation in Canadian provinces, are adopting pesticide restrictions or bans for lawns under certain conditions (Bélair *et al.* 2010). With chemical control options for weeds now limited – by a combination of laws, changing values, and rising costs, effective alternatives for weed suppression are in high demand.

Urban water quality can be negatively impacted by the mismanagement of urban grasslands. Pesticides, including 2,4-D, atrazine, glyphosate, diazinon, and dicambia, have been detected in 25% to 90% of water samples taken after storm events in residential watersheds (Wotzka *et al.* 1994; Schueler 1995; Robbins *et al.* 2001). Common turf pesticides including 2,4-D, mecoprop, pendimethalin, and bifenthrin are mobile both in surface runoff and through soil leachate. These pesticides have been measured above U.S. safety standards in runoff from bluegrass lawns under both irrigated and non-irrigated conditions (Slavens & Petrovic 2012).

Exploring the role of biodiversity in determining an ecosystem's resistance to invasion has been a long studied area within BEF research. Elton's work (1958), is an early example posing a theoretical framework linking diversity and invasion resistance. Over fifty years of research in this area has drawn a few conclusions: a) the species composition of a plant community has an effect on invasion resistance (Crawley *et al.* 1999); b) resource availability, partly influenced by community composition, has an effect on invasibility (Davis *et al.* 2000); c) high niche partitioning in diverse plant communities results in invasion resistance due to complementarity and more complete resource utilization (Tilman *et al.* 1997a; Knops *et al.* 1999; Shea & Chesson 2002). Due to the inherent correlation of these mechanisms (for example changing the species

composition of a community may alter the species richness of the community), determining the contribution of biodiversity alone is frequently confounded (Levine & D'Antonio 1999; Davis *et al.* 2000). Diversity-invasion resistance studies suggest that in general, higher species richness results in a higher probability for a community to resist weedy invasion, *if all other factors are constant* (Hector *et al.* 2002; Hooper *et al.* 2005).

Ecosystem scale matters when considering diversity and invasion resistance. Specifically the point at which biotic "neighborhood" interactions are supplanted by abiotic "regional" controls determines whether diversity suppresses or facilitates invasion (Fridley *et al.* 2007). The *invasion paradox,* articulated by Fridley *et al.* (2007) explores the transition from small-scales (1 m^2) to large-scales (10^7 ha), whereby increasing environmental heterogeneity causes a shift in diversity-invasion likelihood relationships from negative to positive.

Managers of urban grasslands frequently attempt to reduce site heterogeneity through maintenance practices, thereby increasing the potential for plant species richness to have a negative effect on exotic species invasion. Furthermore complementarity in resource utilization, spatially and temporally, may improve a diverse lawn's resistance to invasion. Lastly, sampling effects may increase the probability of including a community member that increases invasion resistance.

Conclusion

Urban grasslands are presently an integral part of developed landscapes across the U.S. and around the world. As native and agricultural ecosystems are converted to simplified turfgrass systems, alterations of ecosystem processes must be quantified in order to prevent the loss of

functionality. We suggest applying the fundamental principles of BEF ecological theory to urban grasslands to improve ecosystem functioning. Specifically, we suggest increasing diversity within urban grasslands to enhance multiple ecosystem services.

Given that the body of BEF research supports the importance of biodiversity and its role in increasing ecosystem functioning, applying BEF theory to urban grasslands is a logical step to enhance the ecological services of these landscapes. It is worth noting environmental heterogeneity of field experiments reduced the magnitude of biodiversity effects, therefore extrapolating BEF research to real-world application may dampen actualized benefits (Loreau *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006). Furthermore, the scale of managed landscapes tends to be negatively correlated with the homogeneity of environmental variables (Fridley *et al.* 2007). Therefore, more *in situ* research is required to determine the extent to which the positive effects of biodiversity can be realized in urban grasslands. Historical BEF emphasis on productivity may be less relevant to turfgrass systems where such increases may require more frequent mowing. Future research should focus on appropriate measures of grassland diversity – α, β, FD, or PD – and ecosystem services relevant to grasslands within the context of urban landscapes. Ecosystem services such as C storage, NO_3^- leaching, invasion resistance, drought tolerance, and disease resistance should be explicitly studied and monitored in urban grasslands. Additionally, studies should be designed to simultaneously measure multiple ecosystem services of interest to increase the multi-functionality of turfgrass polycultures.

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CHAPTER TWO

EXAMINING ECOSYSTEM PROPERTIES IN AN URBAN GRASSLAND BIODIVERSITY GRADIENT

Introduction

Urban grasslands differ from native grasslands or forage-type grasses in assemblage composition and in management techniques. Urban grasslands are turf-type grasses that are semi-regularly mown to a height of 15cm (about 6-inches) or less, may be irrigated, may be fertilized, and are maintained as a ground cover for light traffic, as a sports surface, or for aesthetic reasons (Groffman *et al.* 2009). A range of management practices and intensities are encompassed by this broad definition of urban grasslands; common examples include residential lawns, park landscapes, civic or institutional spaces, and sports fields.

Urban grasslands have expanded rapidly in the United States in recent decades as urbanization has increased. During the recent decadal census, the U.S. Census Bureau (2011) found that over 80% of the nation lives in urban areas. The trend toward urbanization has increased by about 1.8% since 2000. Although lawns comprise only a portion of developed landscapes, altogether they cover more than 2% of the total U.S. terrestrial land area and are three times greater in area than any irrigated crop (Milesi *et al.* 2005).

Converting large expanses of the U.S. to urban grasslands could reduce ecosystem functionality. Negative impacts include a greater potential for non-point source pollution from fertilized lawns, reduced biodiversity, and increased emissions of greenhouse gases from fertilizer applications and mowing practices (Barth 1995; Bormann *et al.* 2001; Guillard & Kopp

2004; Townsend-Small & Czimczik 2010; Raciti *et al.* 2011a). Intensive land management of a monotypic stand of grass may adversely affect, or at least alter, soil biotic diversity and activity compared with more diverse landscapes (Torsvik *et al.* 2002; Clegg *et al.* 2003). As many biogeochemical cycles are dependent upon microbiotic mediation, impacts on soil microbiotic communities should be carefully considered.

To enhance the beneficial ecosystem services of urban grasslands, key drivers must be considered. Terrestrial biogeochemical cycling, especially carbon and nitrogen, are widely impacted by the cultivation of urban grasslands (Qian & Follett 2002; Milesi *et al.* 2005; Pouyat *et al.* 2006; Pouyat *et al.* 2009; Raciti *et al.* 2011b). Additionally, reducing plant diversity in urban grasslands decreases a lawn's natural resistance to weedy invasion (Simmons *et al.* 2011). Management strategies for monotypic landscapes may rely more heavily on weed control via herbicides, contributing to non-point source pollution. Applying ecological theory developed in experimental native grassland ecosystems suggests that increasing plant species richness enhances multiple beneficial ecosystem processes.

Substantial theoretical and experimental work has occurred over the last few decades regarding the functional outcomes of biodiversity in ecosystems. Experimental and observational studies of short and tall grass prairies (native grasslands), drylands, forests, and aquatic ecosystems represent the majority of biodiversity and ecosystem functioning (BEF) research to date (Hooper *et al.* 2005; Balvanera *et al.* 2006; Worm *et al.* 2006; Cadotte *et al.* 2008; Cardinale *et al.* 2011; Maestre *et al.* 2012). Findings from BEF research suggest increasing biodiversity in urban grasslands has the potential to address many of the common environmental issues associated with these ecosystems. To date, biodiversity effects have not been well studied in urban grasslands. A greater understanding of how aboveground urban grassland diversity affects

ecosystem processes will aid land managers in selecting plant assemblages to enhance multiple functions.

The objective of this study is to elucidate the effects of a biodiversity gradient in urban grasslands on multiple key ecosystem processes. From a landscape manager's perspective, altering plant species richness in lawns to optimize beneficial ecosystem services is a feasible practice. However, getting to the stage where research informs practice requires initial studies investigating the effects of biodiversity on ecosystem processes within an urban grassland ecosystem. We conducted an experimental manipulation of richness in turf assemblages at a greenhouse facility to measure the effects of increasing plant diversity on productivity and nitrate leaching. Furthermore we assessed soil bacterial and fungal communities to determine if linkages between plant richness and belowground microbiota may be related to observed biodiversity effects. Based on the literature, we hypothesized that there is a positive, saturating relationship between plant richness and plant productivity. Furthermore, we hypothesize nitrate leaching will decline with increased plant richness. Increasing species richness has been shown to increase productivity, as resources in an ecosystem are more completely utilized (Tilman *et al.* 1996; Cardinale *et al.* 2013). However, abiotic controls ultimately limit the potential productivity of the ecosystem, producing an asymptotic response (Naeem 2002; Hooper *et al.* 2005). Increased resource utilization in more diverse ecosystems reduces the potential leaching of soil nitrate pools (Tilman 1999; Scherer-Lorenzen *et al.* 2003; Mueller *et al.* 2013).

Methods

Greenhouse conditions

Grassland assemblages were grown in mesocosms from October 2012 through May 2013 in a Cornell University greenhouse facility (Ithaca, New York). Greenhouse temperature and supplemental lighting were controlled via an Argus Control Systems (White Rock, British Columbia, Canada) with automated controller sensors suspended above the mesocosms. An array of twenty PL2000 400w HPS lamps by PL Light Systems were used to deliver supplemental lighting daily for 16 hours. See Table 2.1 for environmental data.

Table 2.1 – Greenhouse environmental conditions during the course of the study. Conditions were automatically logged at 15-minute intervals continuously for the duration of the experiment.

	Temp. °C	% Rel. Humidity	CO2 ppm	PAR µmol m-2 s-1
Minimum	12.9	9.4	241.0	0.0
Maximum	31.5	83.2	687.0	1926.0
Average	21.3	44.3	362.6	259.7

Growing media

Topsoil, originating from Genoa, New York, was procured from a landscape supply company. No additional information on soil land use history or soil series is available. Topdressing sand was sourced from the eastern shore of Maryland and consisted primarily of medium and coarse particle size (0.25 to 0.5 mm) with less than 1.5% silt or clay. Soil was mixed in a 2:1 ratio with clean topdressing sand using a Stone Construction Equipment Inc. (Honeoye, NY), Model 950MP concrete mixer. Soil and sand were blended for approximately 5 minutes or until a homogeneous mix was achieved. Blended growing media pH was 8.0.

Mesocosms and grassland assemblages

Thirteen urban grassland assemblages, consisting of six monocultures, three 3-component, three 6-component, and one 12-component polycultures were grown in mesocosms with five replicates each (total n=65). Twelve urban grassland components, available from commercial sources, were selected to construct the mesocosm assemblages. See Table 2.2 for a list of turfgrass components used in this study. The treatment assemblages were selected at random from the available component pool and are detailed in Table 2.3.

Table 2.2 – Urban grassland component pool used in mesocosm study.

Botanical Name	**Common Name**	**Abbreviation**	**Notes**
Festuca arundinacea 'Bullseye'	Tall Fescue	TFBE	a
Festuca arundinacea 'Falcon V'	Tall Fescue	TFF5	a
Festuca ovina var. duriuscula 'Spartan II'	Hard Fescue	HFS2	b
Festuca rubra 'Garnet'	Creeping Red Fescue	CRFG	b
Festuca rubra var. commutata 'Zodiac'	Chewings Fescue	CFZO	b
Festuca rubra var. commutata 'Intrigue 2'	Chewings Fescue	CFI2	b
Lolium perenne 'Amazing GS'	Perennial Rye Grass	PRGA	c
Lolium perenne 'Fiesta 4'	Perennial Rye Grass	PRGF	c
Poa annua var. reptans 'Two Putt'	Annual Bluegrass	ABTP	
Poa pratensis 'Bedazzled'	Kentucky Bluegrass	KBGB	
Poa supina 'Supranova'	Supina Bluegrass	SBGS	
Trifolium repens 'Microgreen'	Microclover	MCMG	
Visual sorting at harvest: a. Tall fescue genotypes were pooled b. Fine fescues pooled c. Perennial ryegrass genotypes			

Table 2.3– Thirteen experimental mesocosm assemblages were randomly selected from the pool of available components. The grassland assemblages consisted of 6 monocultures (treatments 1-6), three 3-component polycultures (treatments 7-9), three 6-component polycultures (treatments 10-12), and a 12-component polyculture (treatment 13). See Table 2.2 for full botanical and common names of component abbreviations used above.

Trt. ID	Co. #1	Co. #2	Co. #3	Co. #4	Co. #5	Co. #6
1	PRGA	-	-	-	-	-
2	SBGS	-	-	-	-	-
3	CRFG	-	-	-	-	-
4	KBGB	-	-	-	-	-
5	MCMG	-	-	-	-	-
6	TFF5	-	-	-	-	-
7	TFBE	KBGB	MCMG	-	-	-
8	HFS2	CFZO	ABTP	-	-	-
9	TFF5	SBGS	MCMG	-	-	-
10	TFF5	HFS2	CFZO	CFI2	ABTP	MCMG
11	CFI2	PRGA	PRGF	KBGB	SBGS	MCMG
12	TFBE	TFF5	HFS2	CFZO	PRGA	PRGF
13	Combination of all 12 components from pool					

The mesocosm containers measured approximately 30 x 38 x 18 cm, and were fitted with custom internal lysimeters to facilitate leachate collection. Lysimeters consisted of a perforated 15 cm long by 1.3 cm outside diameter PVC pipe and a 0.6 cm outside diameter by 60 cm long drainage tube. The pipe was wrapped in filter fabric and installed in the mesocosm so the drainage tube passed through a hole drilled in the bottom of the container. Water-resistant caulk was used to seal gaps around the drainage tube. Mesocosms were filled to within 5 ± 0.5 cm from the top with the growing media and watered until the media settled.

Polyculture seed mixes were blended evenly on a weight basis. Fifty seeds of each type were counted and weighed four times, to determine an average seed weight. Seeding rates were calculated to achieve approximately 3,600 seeds per mesocosm (approximately 3.2 million seeds / 1,000 sf). Prior to seeding, *Trifolium repens* 'Microgreen' was inoculated with D-Nure (INTX Microbials, LLC, Kentland, IN) per the inoculant manufacturer's instructions. Here after, the term grass or turfgrass includes *T. reptans* (a broadleaf legume), unless otherwise stated.

Watering and fertilization

Mesocosms were hand watered with tap water as needed, averaging approximately 2-3 liters per week (0.7-1.0 inch/week) depending on growing conditions. Liquid 21:5:20 (N-P-K) fertilizer was applied at a rate of 200ppm-N from a bulk tank. Fertilizer concentrate was diluted via a Dosatron D14MZ2 14GPM injector by Dosatron International (Clearwater, FL). Fertilizer was applied when growth rates slowed or turf appeared stressed, approximately 1 L every two weeks. Approximately 2.5 g N per mesocosm was added during the course of the experiment, which is nearly equivalent to 4 lbs. $acre^{-1}$ corresponding to a high-end recommendation for Kentucky bluegrass fertilization.

Mesocosm establishment

In October 2012 the mesocosms containing *P. pratensis* and *P. annua* were hand seeded. After bi-directional seeding for even dispersion, seeds were lightly pressed to ensure adequate seed-soil contact. Ten days later, the remaining grassland seeds were added to all mesocosms. After four weeks total, greater than 50% of mesocosms showed greater than 50% germination.

Biomass sampling

Clipping collection began six weeks after initial seeding and continued weekly for seventeen weeks, ending in mid-March 2013. Grasses were mown at a height of 6.5 ± 0.5 cm (2-3 in) and clippings were collected for biomass analysis. Clippings were placed in labeled paper envelopes and dried for 72 hours at 50°C. Samples were weighed directly from the drying oven.

Twenty-three weeks after initiation, one quarter (15 x 19 cm) of each mesocosm was destructively harvested for analyses of standing above and belowground biomass. Grasses were cut at the soil line and samples were dried following the clipping protocol. See below for

rhizosphere soil sampling protocols. Root masses, bulk soil, and unsampled rhizosphere soil were placed in labeled plastic bags and stored at 9° C until the samples could be washed. Roots were isolated by washing samples with tap water and sequential sieving through 4.75 mm (No. 4) and 2 mm (No. 10) standard testing sieves (Advantech, ASTM E-11, USA). Materials passing a 2 mm sieve were discarded. Root samples were dried at 50°C for 72 hours and weighed. Root samples were collected within 7 days of harvest.

An additional one-quarter (15 x 19 cm) subsample of each mesocosm was destructively harvested, keeping root-bound soil mass and aboveground biomass intact. Grasses were carefully separated from the soil mass to keep crowns, tillers, rhizomes, stolons, and roots intact. Grasses were visually sorted into up to seven categories; cultivars of the same species and all fine fescues were pooled, respectively, refer to Table 2.3.

Once sorted, grasses were counted by crown to determine the number of individuals present in the harvested quarter. Daughter plants connected to parent plants by rhizomes or stolons were counted as single individuals. After polycultures were sorted and counted shoots were separated from roots. All materials were dried at 50° C for 72 hours and weighed. Sorted aboveground biomass C and N content were determined using a LECO CN-2000 combustion analyzer (LECO Corporation, St. Joseph, MI).

Rhizosphere sampling

Rhizosphere soil is operationally defined by this study as the soil closely held to roots, which can be freed by mechanical manipulation of the root mass after loose soil has been removed. Soils and root masses from the first destructive harvest were shaken for 60 seconds to free bulk soil. Careful manipulation of soils remaining in the root masses freed rhizosphere soil. Two subsamples (~50 g total) of rhizosphere soil were collected from each mesocosm. Root

masses, bulk soil, and remaining rhizosphere soil were saved for belowground biomass recovery (see above). Rhizosphere soil samples were lyophilized with a Labconco FreeZone 2.5L Benchtop system (Kansas City, MO) and stored at -20° C until RNA extraction.

Leachate samples

Leachate was collected twice during the experiment. Data for the second leaching are reported here. Leaching was conducted by adding 5 liters of water to mesocosms to saturate the soil and cause slight pooling on the soil surface. Lysimeter collection tubes were plugged to allow water to equilibrate with soil pores for 1 hour. Mesocosms were allowed to drain for 1 hour and 50 ml leachate samples were collected. Samples were filtered and stored at -20° C after collection. For leachate analysis, samples were thawed at 4°C overnight and run on an AQ2 Discrete Analyzer by Seal Analytical (Mequon, WI, USA).

DNA extraction, amplification, and T-RFLP

Rhizosphere soil samples were mechanically ground using 13/16-inch cylindrical burundum grinding media (EA Advanced Ceramics, E. Palestine, OH). The MoBio Power Soil DNA Isolation Kit by MoBio Laboratories, Inc. (Carlsbad, CA) was used for extraction of the ground soils. Bacterial 16S rRNA genes were amplified for terminal restriction fragment length polymorphism (T-RFLP) using Bac8F* (5'-AGAGTTTGATCCTGGCTCAG-3') with the 5' end 6-FAM labeled and unlabeled 1492R (5'-CGGTTACCTTGTTACGACTT-3') universal primers. Fungal 18s rRNA genes were amplified using universal fungal primers LROR* (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAAACTTCG-3'). Each 50 µl reaction contained 1.5 µl labeled forward primer, 0.5 µl unlabeled reverse primer, 10 µl Go Taq Buffer, 2.5 mM magnesium chloride, and 2 µl template DNA. Reactions were cycled at 95° C for

3 min, cycled 35 times at 95° C for 30 s, 50°C for 30 s, and 72° C for 45 s, and a 12 min final extension at 72° C (Berthrong *et al.* 2013). Reactions were carried out in duplicate, then pooled.

PCR products were cleaned, concentrated, and desalted via Qiaex II Gel Extraction Kit (150) by Qiagen Technologies (Germantown, MD) with repeated elution to increase yield. DNA concentration was quantified by using Quant-iT PicoGreen dsDNA Kit by Invitrogen (Carlsbad, CA) using a 96-well BioTek Synergy HT luminescence microplate reader with Gen5 software for data collection and analysis (Winooski, VT). Cleaned PCR products were normalized to achieve a DNA template target concentration of ~350 ng per 15 µl reaction for the enzymatic digest. Digestion reactions were carried out using the *Hae*III restriction enzyme with CutSmart Buffer. Cleaned digests were dried in a rotary evaporator and resuspended in 9.7 µl formamide and 0.3 µl of 500 LIZ size standard (Applied Biosystems). Terminal restriction fragments (TRFs) were quantified with a 3730XL gas capillary auto analyzer (Applied Biosystems) following the EPA-114-A Rev. 8 method. See Liu, Marsh et al. 1997 for more details on T-RFLP analysis.

Statistical analysis

To test the effect of diversity-level treatments on response variables, ANOVA was run using JMP Pro 10 (SAS, Cary, NC). Transformations of data were made as necessary to fit model assumptions of normality and constant variance. Tukey's Honestly Significant Difference (HSD) tests were used to determine significant differences between diversity effects ($p < 0.05$). All figures presented in this manuscript present means and standard errors of raw data scales and statistical analyses of transformed data, unless otherwise noted. Analyses of the T-RFLP data for this study are based on protocols described in Berthrong et al. (2013) and Abdo et al. (2006), with the modifications noted below. Electropherograms were analyzed and compared to size standards using Peak Scanner ver. 1.0 (Applied Biosystems). Bacterial peaks were analyzed

within the standard (50-800 bp) and above 50 units of height. Fungal peaks outside of the standard and below 200 units of height were excluded. Peak height parameters for fungal samples were selected to meet data input parameters of PC-Ord. The online T-Rex software filtered noise, clustered, and aligned peaks in order to determine the TRFs present. TRF peak area was averaged over two laboratory replicates and relitivedized within samples for the bacterail and fungal samples separately. TRFs not appearing in greater than 3 samples were omitted. PC-Ord ver. 5.31 was utilized to perform non-metric multidimensional scaling (NSM) to visualize similarities within the TRF data. A squareroot transformation was applied to both the bacterial and fungal data to reduce apparent variability for analysis. Bacterial samples utilized a Relative Sorensen distance measure to achieve the lowest model stress and produce a 2-dimensional interpretation. Fungal samples were analyzed with a correlation distance measure resulting in a 3-dimensional model with the lowest stress. Outliers exhibiting greater than two times the standard deviation of the respective distance measures were detected in both the bacterial and fungal analyses. Four outliers were removed from bacterial analyses as the samples were not correlated with specific treatments. Three of five outliers for fungal analyses were related to the monoculture *Trifolium* treatment, thus ouliers were included in the analyses. The Shannon indices (*H'*) of soil TRF diversity for bacterial and fungal communities were calculated using the online EstimateS software (Robert K Colwell software, Stoors, CT). Data was scaled one hundred-fold to produce integer abundance values for *H'* calculations.

Results

Total aboveground productivity increases with diversity

Clipping productivity, summed by replicate, for 17 weekly samplings, did not result in significant differences between monoculture and polyculture treatments ($p=0.13$, $R^2=0.09$). An apparent, but not significant trend indicated enhanced biomass as diversity increased across all treatments (Figure 2.1). Standing aboveground biomass from the first harvest date shows the 3-component polycultures were significantly higher from monoculture treatments, however differences were not observed at 6- and 12-component polycultures ($p=0.0038$*, $R^2=0.20$) (Figure 2.2). Belowground biomass analyses from the first harvest did not yield significant differences at any diversity treatment level ($p=0.81$ $R^2=0.02$) (Figure 2.3). Total aboveground productivity (clippings + standing biomass) was positively associated with diversity level and significantly different between monoculture treatments and 3-and 6-component polycultures. Greater replication of 12-component polycultures would have resolved significant differences between mono- and polyculture treatments (Figure 2.4). Where possible, grassland mesocosms were tested for species effects by treatment. Figures for clipping productivity (Figure A. 1), aboveground biomass at harvest (Figure A. 2), the partitioning of aboveground biomass by visual component sorting (Figure A. 3), and total aboveground productivity (Figure A. 4) analyzed by treatments are presented in the Appendix. In polycultures containing *Poa annua var. reptans* and *P. supina*, mean standing biomass consisted of 17.4 – 87.4% and 38.8 – 58.0% of total aboveground biomass, respectively (Figure A. 3). In one 3-part polyculture treatment, *P. annua* produced 88.3 g of biomass at harvest, which nearly equaled the most productive monoculture treatment *P. supina* that produced 89.6 g (Figure A. 3 and Figure A. 4). It is possible that if *P. annua* had been included in monoculture treatments the average treatment response would have

increased, nevertheless many of the species not included in monoculture were not highly productive and would have moderated or reduced the average monoculture productivity.

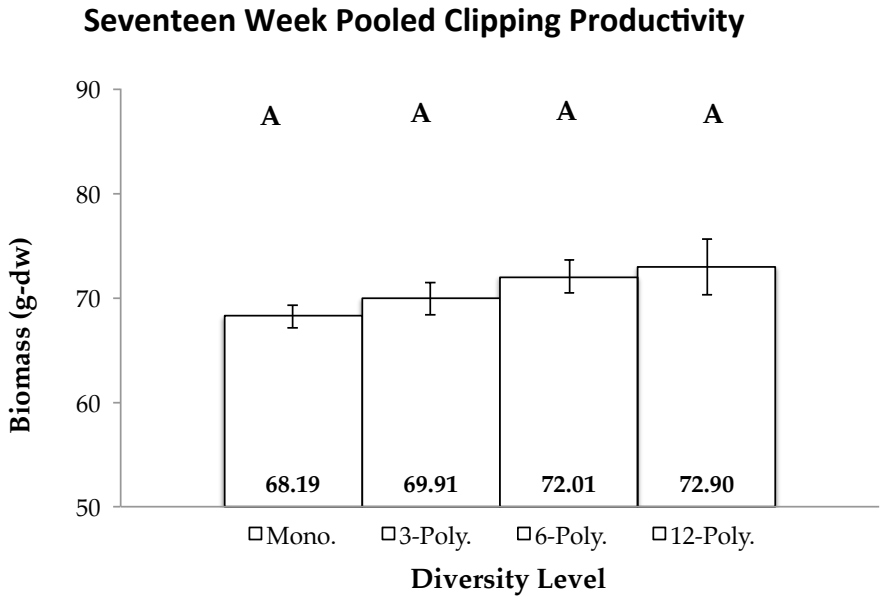

Figure 2.1 –Weekly clipping productivity in grams dry weight was summed over seventeen weeks. Clipping productivity suggests a slight positive trend with increasing diversity, but the trend was not significantly different between monoculture and polyculture treatments using a Tukey test ($p=0.13$, $R2= 0.09$). Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

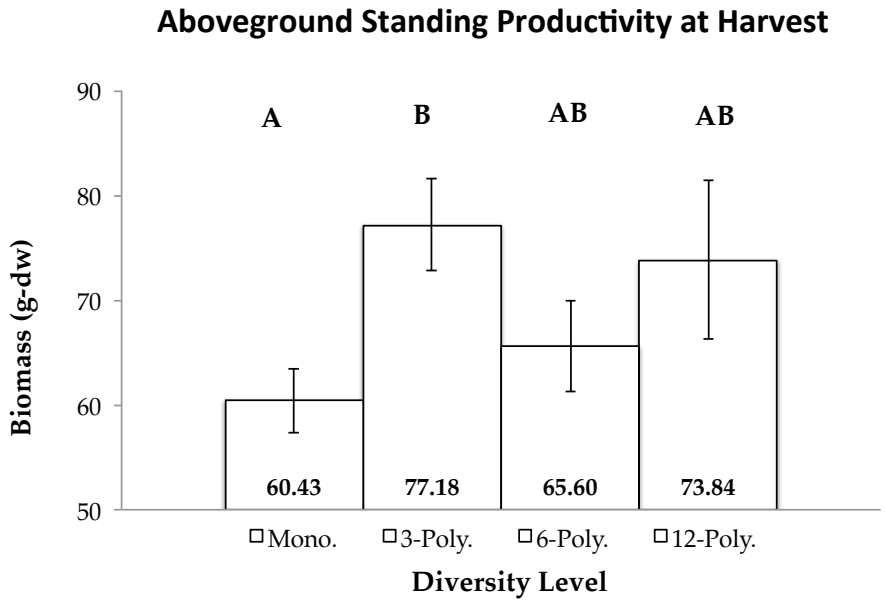

Figure 2.2 – Standing aboveground biomass in grams dry weight was significantly different between monoculture and 3-component polyculture treatments, but 6- and 12-component polycultures were not distinguishable from either the monoculture or 3-part polyculture treatments via a Tukey test ($p=0.0038^*$, $R^2=0.20$). Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

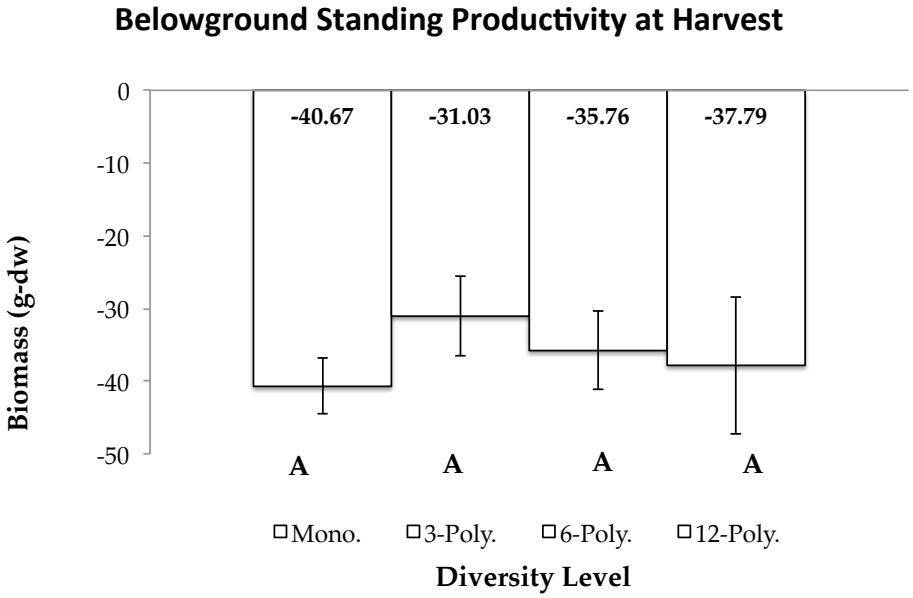

Figure 2.3 - Belowground productivity, denoted by negative values, was not significantly different among diversity treatments ($p=0.81$, $R^2 = 0.02$). Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

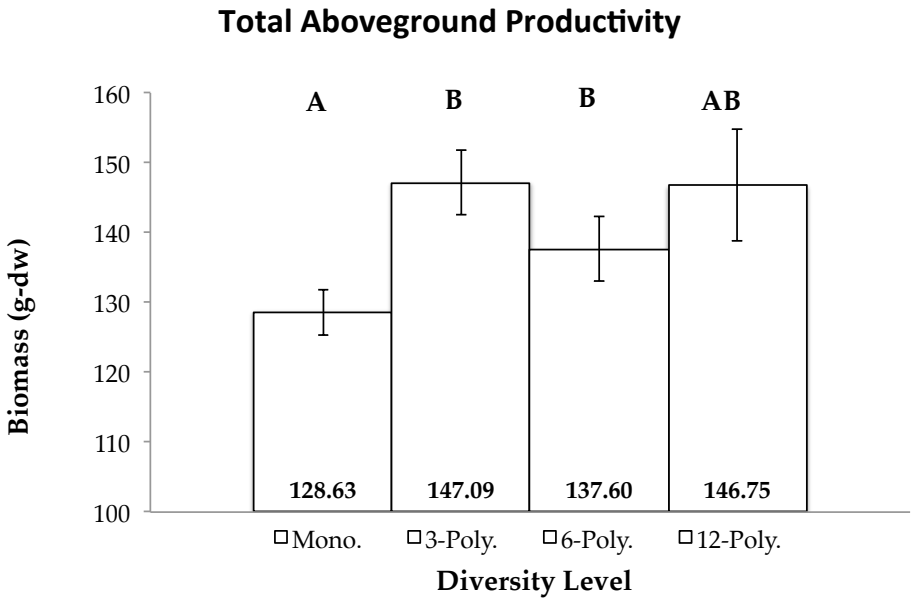

Figure 2.4 – Total aboveground productivity (clippings + standing biomass) was significantly different between monoculture and 3- and 6-component polycultures ($p=0.0059$, $R^2=0.18$). Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

Nutrient leaching is reduced with increasing plant diversity

Nitrate (NO_3^-) concentration (mg/L) in leachate diminished significantly on average in the 3- and 6-component polycultures compared to the monoculture treatments ($p=0.0028$*). The 12-component polyculture also showed reduced NO_3^- leaching, however variability and low replication did not allow for statistical differentiation from monoculture treatments (Figure 2.5). Combustion analysis of harvested aboveground biomass for N content (Total N g) revealed a slight positive, but non-significant trend ($p=0.0816^{\dagger}$, Figure 2.6). Leachate NO_3^- concentrations were compared by treatment to determine species effects where possible, refer to Figure A. 5 in the Appendix. Of the monocultures, *Festuca arundinacea* 'Falcon V' (TFF5) resulted in the lowest mean nitrate leaching, while *Trifolium repens* 'Microgreen' (MCMG) had the highest (5.64 ± 0.69 and 10.70 ± 0.69, respectively on square root transformed data with a pooled

standard error estimate). Polyculture treatments containing TFF5 (12, 13) were significantly lower than other polyculture treatments. However, when MCMG was included with TFF5, the polyculture treatments (9, 10) the mean leaching increased and was not significantly different than other polyculture treatments. Furthermore, the lowest overall mean leaching (4.90 ± 0.69, square root transformed data with a pooled error estimate) was observed in treatment 8. No components of treatment 8 were grown in monoculture for comparison.

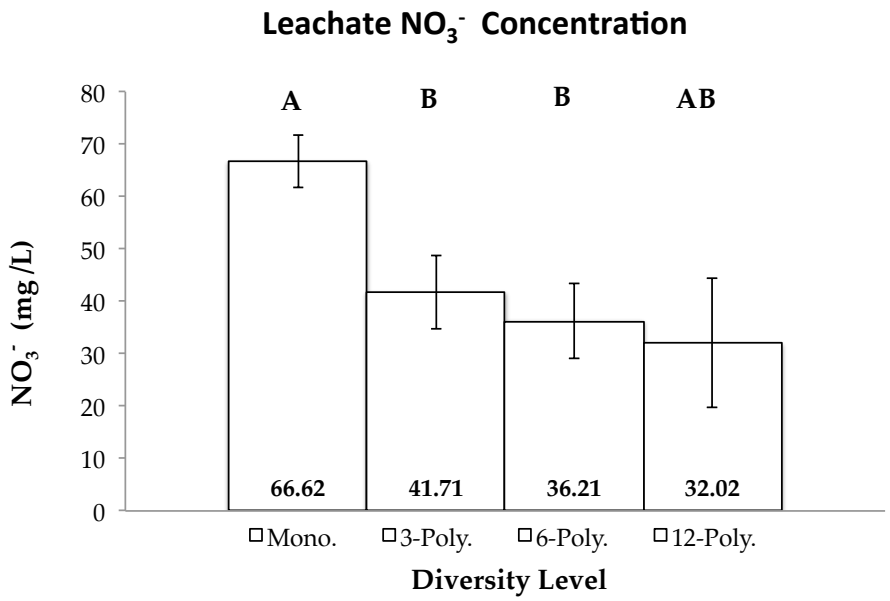

Figure 2.5 – Leachate NO_3^- (mg/L) concentration decreased from monoculture through 6-component polyculture treatments. The 12-component treatment continues the decreasing trend and higher treatment replication would have allowed the resolution of significant differences from monocultures ($p=0.0028^*$, $R^2=0.20$). Letters show significant differences according to a Tukey test. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

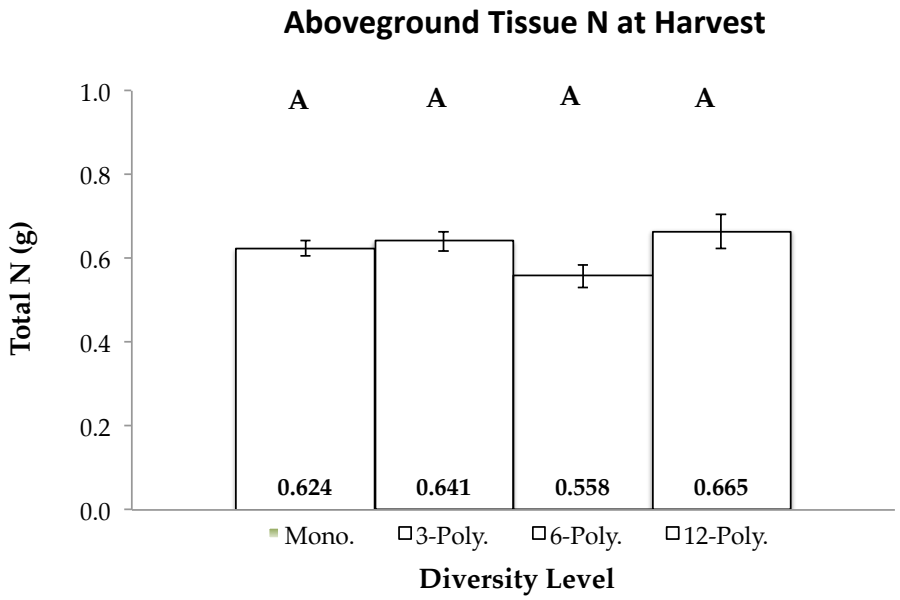

Figure 2.6 – Aboveground tissue N at harvest was not significantly different across diversity treatments ($p=0.0816^{\dagger}$). Letters indicate results from Tukey test. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

Convergence of bacterial community structure with increasing plant diversity

T-RFLP data were used to assess the relative structure of soil bacterial and fungal communities in the urban grassland mesocosms. NMS analysis of Bacterial 199 TRFs using a square root transformation and a Relative Sorensen distance measure resulted in the lowest stress (9.27) and a 2-dimensional model solution that accounted for 94.3% of the variability observed in samples. Soil bacterial communities under monoculture conditions show higher variability in community composition, however large portions of bacterial communities are shared regardless of diversity treatment. Variability in the community structures of the 6- and 12-part polycultures are similarly aligned (Figure 2.7).Fungal TRFs used a correlation distance measure for NMS analysis upon squareroot transformed data to create the lowest stress model (14.09) and a 3-dimensional solution accounting for 87.5% of the observed variation. NMS fungal data are presented in one biaxial plot (axes 2 and 3) which show the greatest differences in community

variability (Figure 2.8). There is no underlying structure to the fungal communities in relation to diversity level treatments.

Soil microbial diversity increases within multiple-species grasslands

Shannon's diversity (*H'*) for soil bacterial and fungal TRFs were calculated and are presented in (Figure 2.9 and Figure 2.10 respectively). Fungal relative abundance, as determined from peak florescence, was weaker compared to bacterial samples. Fungal abundance was amplified a hundred fold to resolve differences in community diversity, thus interpretations of fungal richness must be made with caution. Bacterial communities in monoculture treatments displayed reduced richness compared to the polyculture treatments. Furthermore, the diversity of the 3- and 6-component polycultures was indistinguishable. No significant differences were found between the 12-component polyculture and the other polyculture treatments. As with bacterial community richness, fungal richness increased in response to increasing plant diversity from one to multiple species. Low statistical power at the highest diversity treatment resulted in the in ability to detect differences in bacterial and fungal richness.

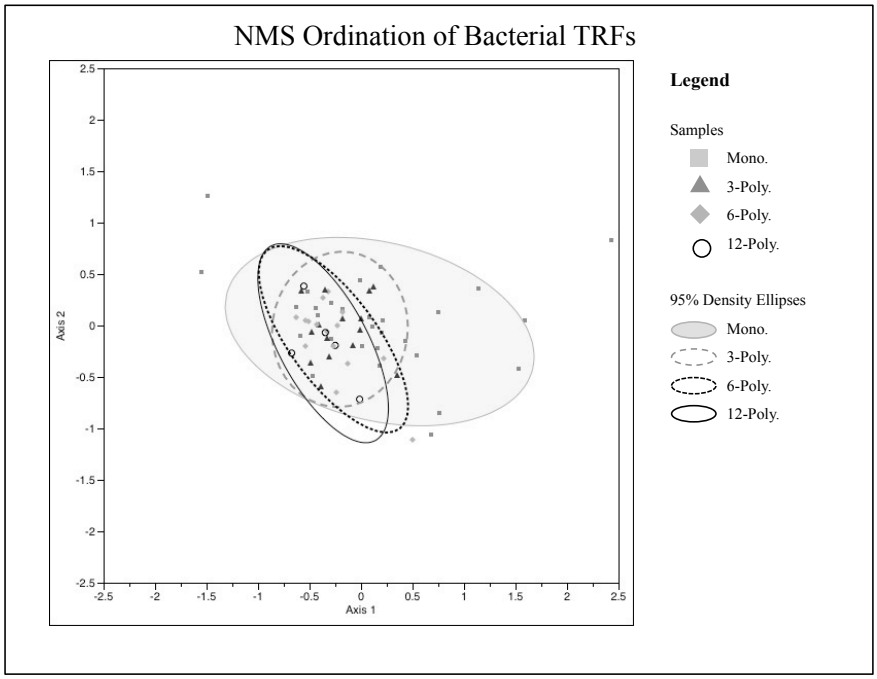

Figure 2.7 – NMS ordination of bacterial TRFs show high variability among monoculture treatments and large portions of overlap between treatments. Bacterial community structure is most similar at the 6- and 12-component diversity level treatments. Axis 1 R^2=0.888 Axis 2 R^2=0.055, cumulative R^2=0.943.

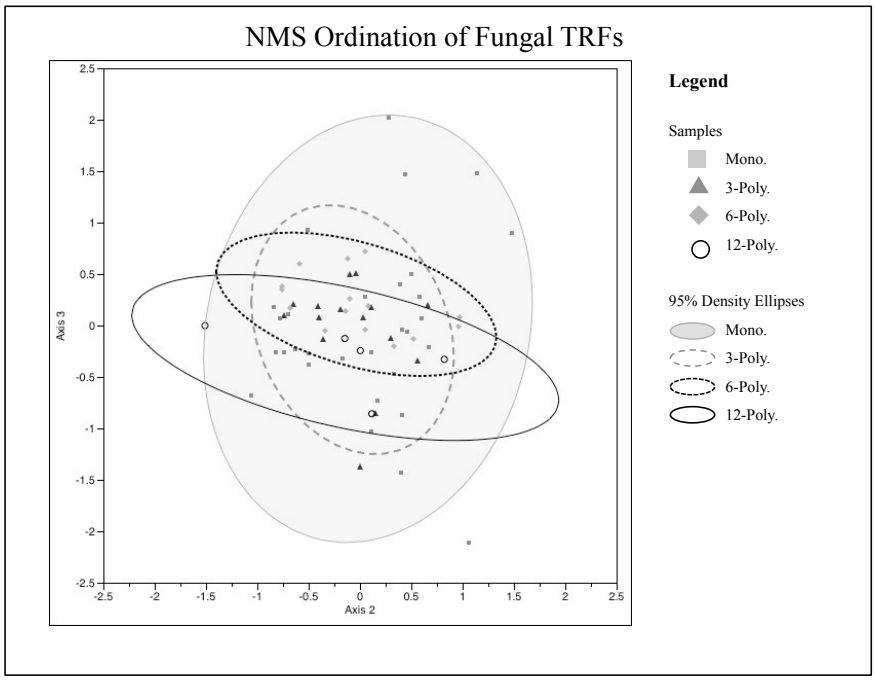

Figure 2.8 – NMS ordination of fungal TRFs shows high variability at all treatment levels and little distinguishable pattern. Axis 1 R^2=0.245 (not shown), Axis 2 R^2=0.257, Axis 3 R^2=0.372, cumulative R^2=0.875

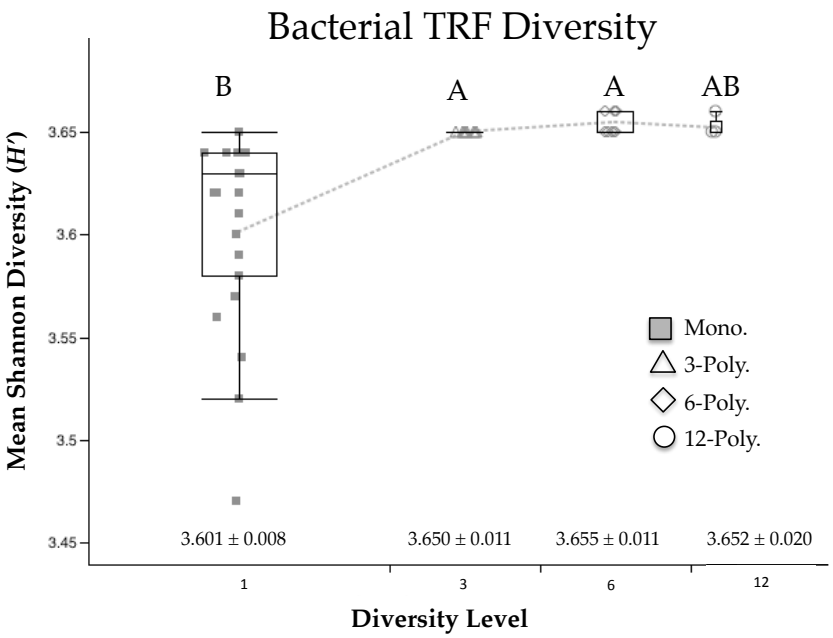

Figure 2.9 – Soil bacterial microorganism richness, as measured by mean Shannon diversity (*H'*), increased significantly in response to aboveground plant diversity. Letters indicate significant differences via Tukey test. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

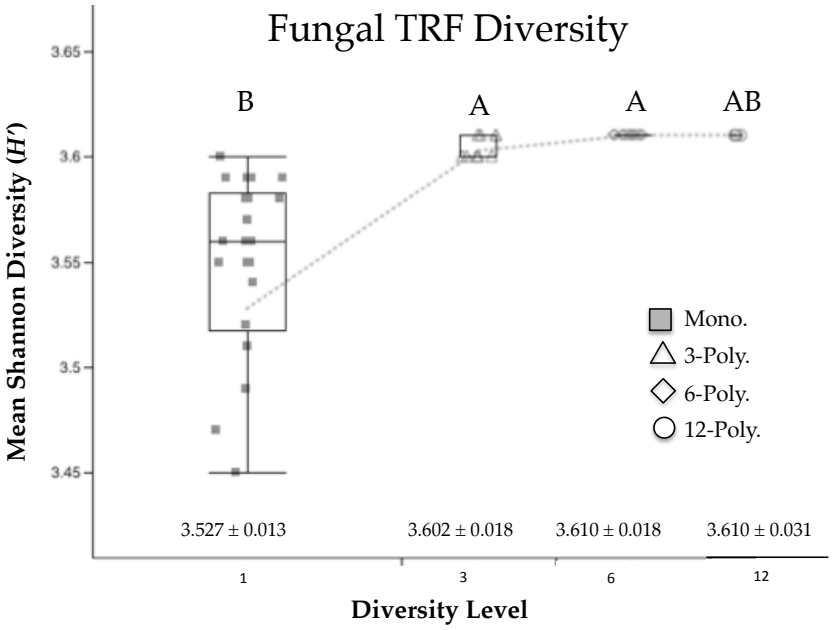

Figure 2.10 – Soil fungal microorganism richness, as measured by mean Shannon diversity (*H'*), increased significantly in response to aboveground plant diversity. Letters indicate significant differences via Tukey test. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

Discussion

Research over the past few decades has sought to study the effects of plant species diversity on ecosystem processes. Biodiversity effects have not previously been explicitly studied within the context of urbanized landscapes, such as urban grasslands. We examined how productivity and nitrate leaching are modified when plant diversity increases in experimental grassland mesocosms to determine if ecological theories regarding the importance of biodiversity applied to manage urban landscapes. We found increasing plant richness increases total aboveground productivity and reduces nitrate loss. Not only were ecosystem processes altered, but changes in ecosystem properties, bacterial and fungal community structure, were also observed. This study shows the utility of using mesocosm studies prior to field experiments as near-significant trends for productivity and retention were observed at the highest diversity level, yet high variability and low replication resulted in low statistical power to detect treatment differences (Ott & Longnecker 2008). Thus preliminary data generated from mesocosm research can inform future field-scale experiments buy showing the need for increased replication at least two-fold among the highest diversity treatments.

Productivity increases with diversity, but may be driven by sampling effect

Total aboveground productivity (standing + clippings) increased significantly from monoculture to polyculture treatments. Though there was a wide range in species-specific productivity, this indicates certain species may be highly productive, even in polyculture. Interestingly, though *P. supina* and *P. annua* were highly dominant in monoculture or 3-polyculture, respectively, they were not as productive or dominant at higher diversity levels (Figure A. 3). Controlled manipulations of grassland diversity and composition have also concluded the identity of species present may be as important as functional richness in

determining effects on ecosystem processes (Hooper & Vitousek 1997; Hooper 1998; Scherer-Lorenzen *et al.* 2003). This finding supports the *sampling effect* hypothesis for describing biodiversity-productivity effects. It proposes that higher diversity assemblages have a greater likelihood of including a highly productive species, thus on average a polyculture is more likely to be highly productive than an average monoculture (Aarssen 1997). Despite not having all treatments in monoculture for comparison, biodiversity effects are apparent, even if differential sampling of the species pool is mechanistically driving observed diversity effects.

Belowground productivity was found to be uncorrelated (R^2=0.02) with plant diversity, which conflicts with BEF trends in native grasslands (Hooper *et al.* 2005; Pasari *et al.* 2013). This finding may be an artifact of sampling methods or experimental design. Mesocosm size was selected in order to facilitate high-diversity polycultures, yet container depth (~18cm) may have been insufficient to allow complete root-system development (Turgeon 2005; McCarty 2011). Evidence of this was apparent upon destructive harvest when a majority of samples were root-bound. Alternatively, root-sampling procedures may have resulted in a larger proportion of fine root loss. Although fine roots represent a large proportion of turfgrass fibrous root systems, the low dry weight of fine roots would likely not have biased analyses significantly. Losses in fine root recovery were assumed to be equal across all treatments and therefore comparison of remaining dry root biomass is valid.

In the context of urban grasslands, aboveground productivity is a two-sided issue. High productivity is desired to i) quickly establish a closed turf stand to suppress weedy invasion; ii) enable the turf to grow at a sufficient rate to overcome maintenance-associated stresses (i.e. trafficking and mowing); iii) outcompete fungal and bacterial pathogens (Turgeon 2005; McCarty 2011). However, increased productivity also increases the frequency of mowing to

maintain turf at a desired height. More frequent mowing has environmental impacts stemming from the burning of fossil fuels and economic consequences from labor, fuel, and repair costs (Zhang *et al.* 2013a). This experiment suggests that on average, even moderate increases in diversity may result in minor increases in urban grassland productivity compared to monoculture treatments. While the contributions of all species included in this study were not individually measured, the collective response does underscore the role of plant species diversity in enhancing productivity.

Nitrate leaching reduced under diverse polyculture

As was hypothesized, plant diversity was negatively associated with nitrate leaching. Leachate samples were shown to contain lower concentrations of nitrate (NO_3^-) as diversity increased from monoculture to 3- and 6-component polycultures ($p=0.0028^*$, $R^2=0.20$). As with aboveground productivity measures, increased replication at the highest diversity level would have shown significant differences versus monoculture treatments. Tilman *et al.* (1996) found root-zone extractable NO_3^- across a native grassland (prairie) diversity gradient to be negatively correlated with diversity ($R^2=0.22$) and reached a minimum between 8-12 species, beyond which NO_3^- concentrations were not observably different. Similarities between Tilman *et al.'s* findings and our study for the strength of correlation and plant diversity levels provide strong evidence that aboveground diversity does reduce NO_3^- leaching in urban grasslands. When considered on a species basis, our findings concur with other studies that have found polyculture composition may be as important as richness in determining ecosystem function (Hooper & Vitousek 1997; Tilman *et al.* 1997a; Scherer-Lorenzen *et al.* 2003; Hooper *et al.* 2005). While individual species traits, interspecific competition, and complementarity should be considered, as has been

suggested (Aarssen 1997), diversifying a plant community increases the likelihood of including influential species.

Retained NO_3^- may be explained by enhanced plant uptake, incorporation into microbial biomass, gaseous loss (NH_3, N_2O, or N_2), or a combination of these processes (Schimel & Bennett 2004; Mueller *et al.* 2013). The experiment was not designed for determining a complete N budget, however post hoc combustion analyses were performed to determine N content of aboveground standing biomass. Total tissue N (g) trended towards significance ($p=0.0816^{\dagger}$) and increased with plant diversity. However, tissue N and productivity alone do not account for the increased NO_3^- retained under higher diversity treatments. While belowground N content was not measured, soil microbiota are known to regulate the cycling of N soil pools through enzymatic activities and symbiotic associations (Kaye & Hart 1997; Schimel & Bennett 2004; Van Der Heijden *et al.* 2008). Microbial biomass may account for the observed NO_3^- retention and should be included in future studies. Furthermore, some grass species, including perennial ryegrass, have been shown to produce nitrification inhibitors in the rhizosphere that may reduce nitrates available for leaching (Zhang *et al.* 2013a).

It is well known that N-fixing plants can have extremely different impacts on NO_3^- leaching vs. non-fixing plants (Scherer-Lorenzen *et al.* 2003). Two studies of species and functional group diversity effects in native grasslands found legumes were responsible for the greatest NO_3^- leaching and polycultures containing legumes leached more than non-legume containing mixtures (Scherer-Lorenzen *et al.* 2003; Mueller *et al.* 2013). These findings are consistent with our observations of *T. repens* 'Microclover' in mono and polyculture treatments (Figure A. 5). This finding does support species-specific effects on assemblage NO_3^- leaching

results, but also reinforces the value of diversity in limiting NO_3^- leaching through *complementarity* and *sampling effects*.

Urban grasslands have shown great potential for N retention (Gold *et al.* 1990; Raciti *et al.* 2008). Yet, management practices, landscape slope, irrigation and fertilization rate, and lawn age can alter urban grasslands status as N sources or sinks (Groffman *et al.* 2009). If results from this study are confirmed in the field, it suggests that grassland biodiversity can be manipulated to assist in NO_3^- management, reducing contributions toward the eutrophication of urban watersheds. More study is required to elucidate if positive biodiversity-nutrient retention effects occur when urban grassland communities experience typical *in situ* stresses.

Aboveground diversity alters ecosystem properties by affecting microbial diversity and structure

Soil bacterial and fungal community structures were evaluated using DNA amplification T-RFLP methods. Rhizosphere bacterial community composition was shown to be highly variable among monoculture treatments, resulting in different community compositions that observed in polyculture rhizospheres. Soil fungal communities were highly variable and thus were not able to be well characterized in response to plant diversity treatments. Despite difficulties in discerning structural differences in rhizosphere microorganism communities, our findings do provide evidence for a link between plant species richness and microorganism richness and evenness as measured by the Shannon index. While TRF diversity does not correlate directly with true microbial diversity, it can give a reasonable estimate in communities with reduced complexity (Blackwood *et al.* 2007). Managed ecosystems tend to have lower diversity soil microbial communities when compared to natural environments (Torsvik *et al.* 2002; Berthrong *et al.* 2013), therefore *H'* is a reasonable diversity measure for this study. Our study found increasing plant richness might have significant impacts on soil microbial richness.

This finding concurs with results discussed in Kowalchuk *et al.* (2002), which found positive linkages between plant richness and soil microbial diversity. Kowalchuk *et al.* noted macrophyte diversity effects were limited to the rhizosphere and bulk soil bacterial diversity was typically higher than the rhizosphere.

Changing land uses from unmanaged natural landscapes to managed urban or peri-urban grasslands can alter soil microbial communities and associated biogeochemical processes. Generally, reductions in soil microbial diversity are associated with high disturbance, managed ecosystems (e.g. agricultural and urban) (Torsvik *et al.* 2002). Indeed Webster *et al.* (2002) noted reductions in the heterogeneity of soil characteristics and ammonia oxidizer diversity under grassland irrigation and fertilization management regimes. Yao *et al.* (2006) found land use change from pine forest to turfgrass resulted in shifts in microbial community structure in as little as one year. Increasing urban grassland diversity may offer one means for offsetting potential negative consequences for soil microbial diversity. Soil microbiota, particularly mycorrhizal fungi in grasses, are vital for the mineral nutrition of an urban grassland (Malinowski & Belesky 2000). A suite of soil microorganisms are responsible for the cycling of organic nitrogen and phosphorous to available inorganic forms through an array of exoenzymes and metabolic processes (Schimel & Weintraub 2003; Schimel *et al.* 2005). Reduced soil biotic diversity can negatively impact plant community structure and function (Van Der Heijden *et al.* 2008). Consequently, future studies should probe the strength of linkages between plant diversity and rhizosphere microbial community structure. Specifically, research should focus on microbial community effects that drive differences in plant performance.

Species effects and other diversity measures may be relevant in urban grasslands

This study indicates, at a mesocosm scale and under controlled conditions, biodiversity effects are present in anthropogenically-influenced urban grasslands. However, *in situ* experiments manipulating urban grassland diversity are needed to determine if, and to what magnitude, biodiversity effects occur when growing conditions are less controlled. Species effects are likely to exist which are correlated with diversity effects. However, species effects can only be determined with regard to a relevant ecosystem function. Here, two *Poa* species were highly productive and *Trifolium repens* influenced NO_3^- leaching. These species may be less influential in the context of a different ecosystem function. Therefore, regardless of uncertainties concerning biodiversity effects, there appears to be no evidence suggesting reducing biodiversity benefits ecosystem services. Furthermore, this study suggests plant biodiversity can have multiple benefits when the multi-functionality of an ecosystem is considered.

Within the last ten years BEF literature has attempted to refine the mechanistic understandings of plant trait differences leading to *niche differentiation*, *complementarity*, *sampling effects*, and community ecosystem functioning. Assemblage species richness has been shown to be a good predictor of productivity, but functional diversity (FD) and phylogenetic diversity (PD) have been shown to be better predictors in experimental native grasslands (Cadotte *et al.* 2008; Cadotte *et al.* 2009; Flynn *et al.* 2011). The utility of PD as a predictor of function is less applicable to landscape managers and habitat restorationists than species richness, particularly since species number and PD have been found to be highly correlated (Cadotte *et al.* 2009). Furthermore, PD estimation relies on the availability of genetic sequences to construct phylogeny, which is often unavailable or relies on assumptions about congeneric

relatives. Lastly, FD may be important, particularly for legumes as predictors of productivity or nitrate leaching, but functional group importance is related to the ecosystem function in question. Increasingly as multi-functionality is emphasized, defining relevant *a priori* functional groups will become more complex. Therefore, richness as a first approximation of biodiversity supporting ecosystem function is useful, especially for professionals charged with actively managing landscapes.

Conclusions

Urban grasslands have traditionally reduced the diversity of the native or agricultural landscapes, which they replace. Such reductions in species richness have been known to have negative consequences for ecosystem functioning. Our research show simple mesocosm manipulations of grassland richness can concurrently enhance productivity, decrease nitrate leaching, and improve soil microbiotic richness. This study also emphasizes the importance of gathering preliminary data with mesocosms before implementing field-scale experiments since trends were observed in both ecosystem processes and properties that can be further tested with slight experimental design modifications in future research. Where aesthetic and surface-tolerances of urban grasslands allow, a diverse turfgrass community may offer multiple beneficial ecosystem functions. Municipal parklands, commercial landscaping, institutional green spaces, and residential lawns would be ideal sites for increasing plant diversity of existent and proposed grasslands for the express purpose of improving urban ecosystem functioning.

As we have shown, ecological theory derived from natural ecosystems is applicable to urban environments. Where possible BEF theory and other ecological theories should be applied to developed landscapes in order to maintain or increase ecosystem services otherwise degraded

during the process of urbanization. Furthermore, a wider array of turfgrass species, cultivars, and functional type combinations should be evaluated for biodiversity effects. Restoration efforts should utilize plant richness in rehabilitating sites to achieve greater functionality, particularly in urban landscapes that have previously been overlooked in regards to ecosystem function and current ecological theory.

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APPENDIX

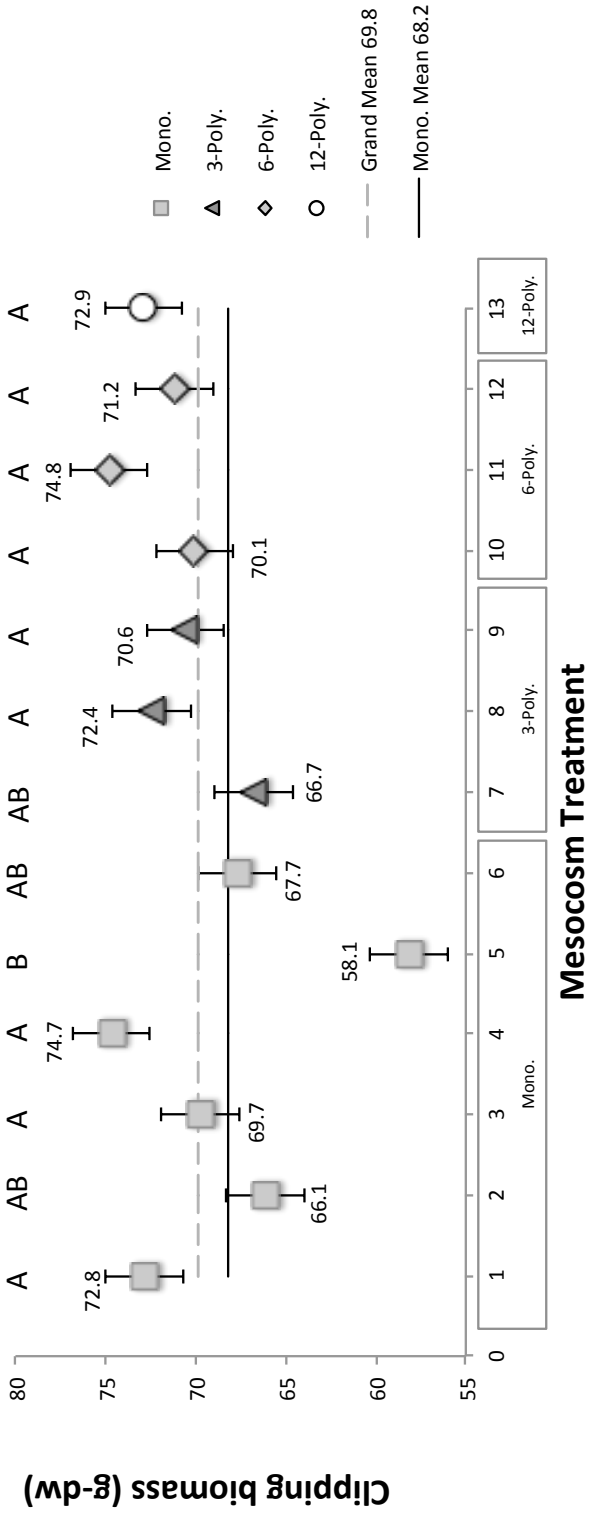

Figure A. 1– Clippings were collected and pooled for seventeen weeks and compared across mesocosm treatments to determine weekly productivity. Treatments are grouped by diversity level for reference. Values are presented for treatment means, grand mean, and mean monoculture productivity. Letters indicate significant differences between treatments when monocultures are compared alone via a Tukey test. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

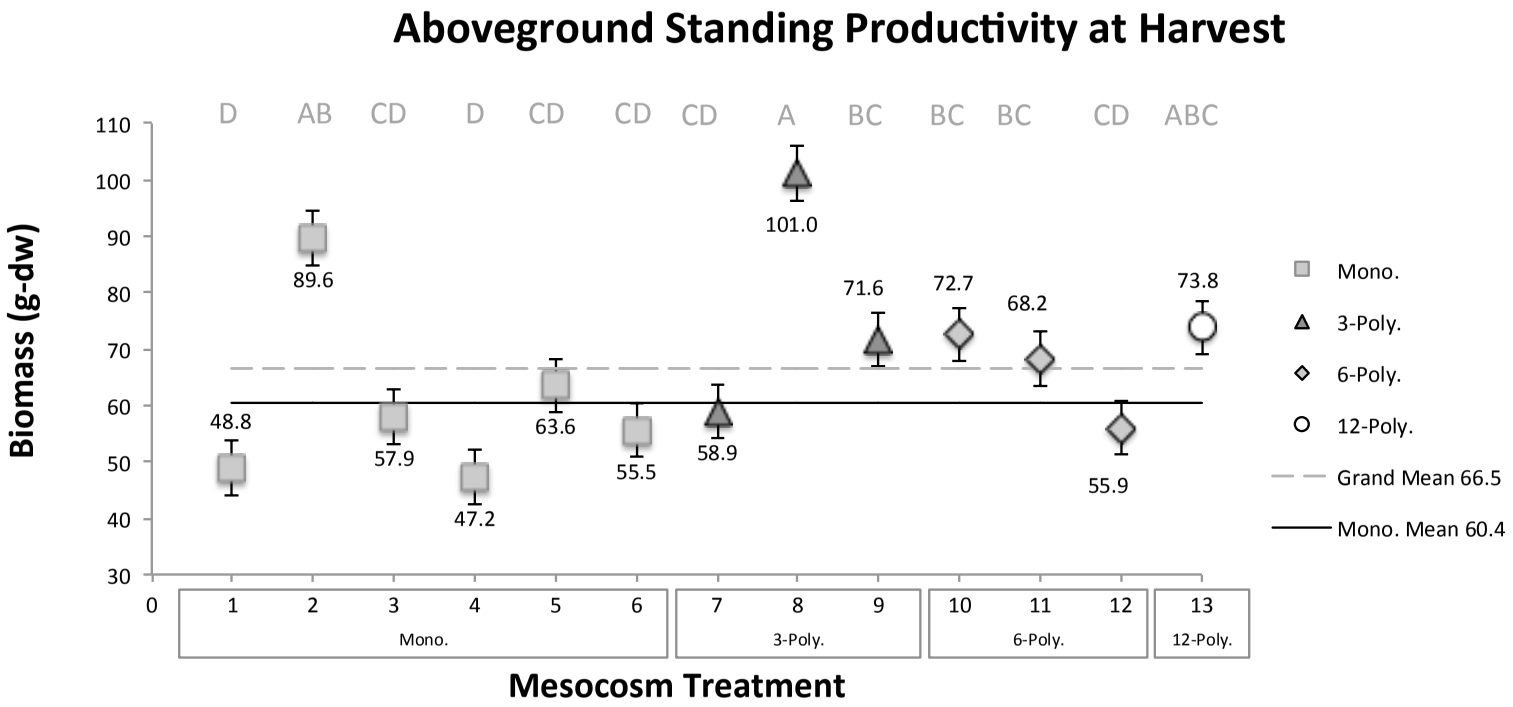

Figure A. 2 – Standing aboveground productivity at harvest (g –dw) shows species effects among monoculture treatments. Grey letters indicate significant differences via a Tukey test. *Poa supina* 'Two Putt', treatment 2, was more productive than the other monoculture treatments. Treatment 8 was the most productive treatment and contributes greatly to the overall response of 3-part polycultures. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

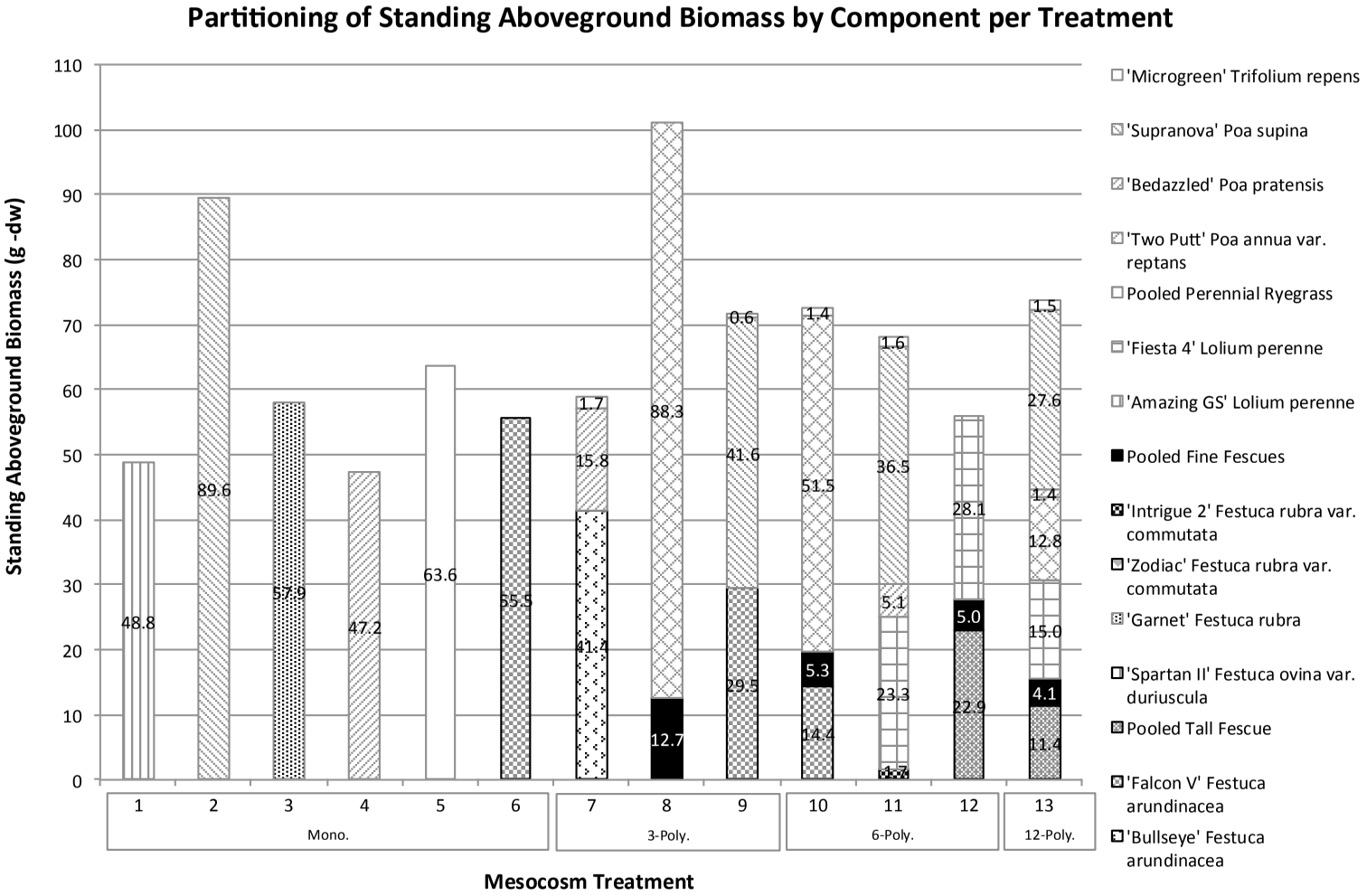

Figure A. 3 – Partitioning of the standing aboveground biomass by visual sorting of community components indicates potential species effects. *Poa annua*, represented nearly 87% of the productivity of treatment 8, suggesting it may have been highly productive if grown in monoculture. Furthermore, *P. annua* in treatment 8 produced a similar standing biomass as *P. supina* in monoculture. However, *P. annua* was not as dominant in 6- and 12-component polycultures. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

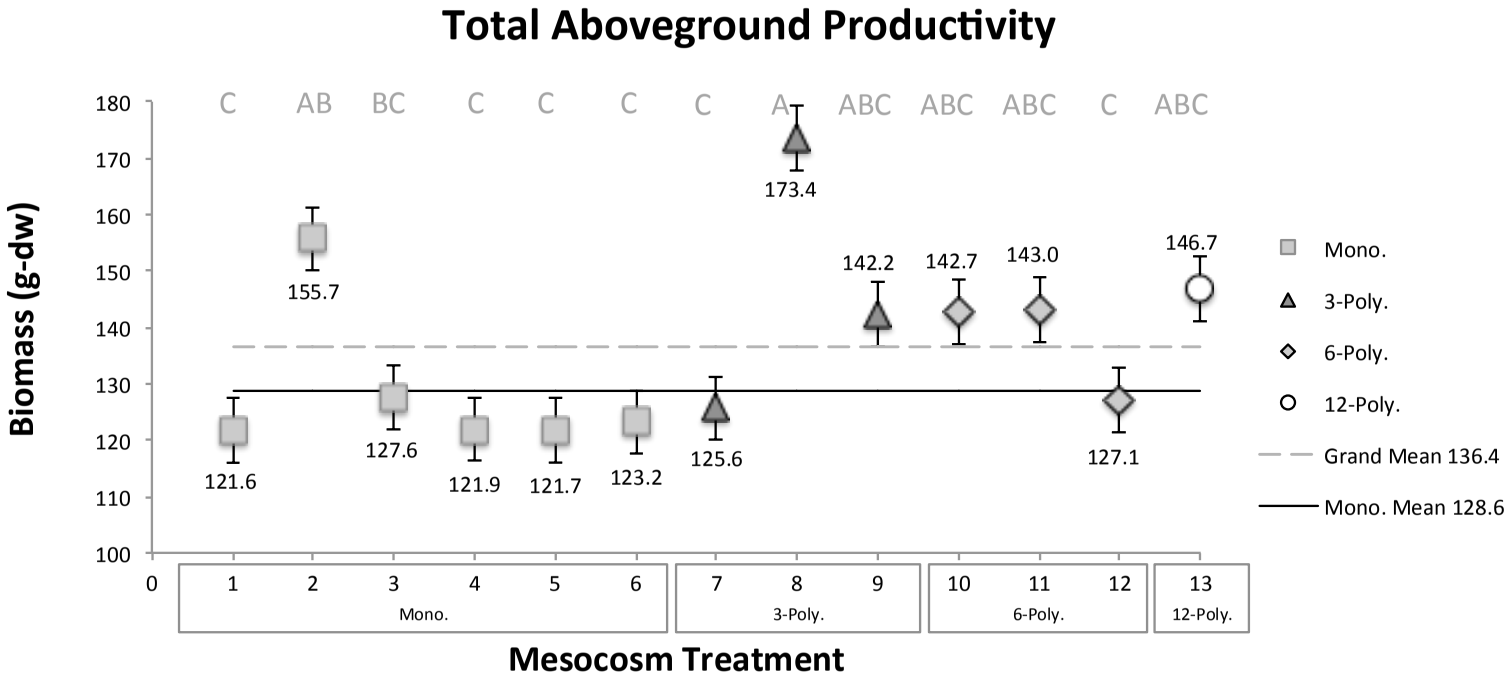

Figure A. 4 – Total aboveground productivity by treatment indicates significant species effects among monoculture treatments via Tukey's HSD test (grey letters). *Poa supina* was the most productive species when standing aboveground and weekly clippings were pooled and measured. Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means.

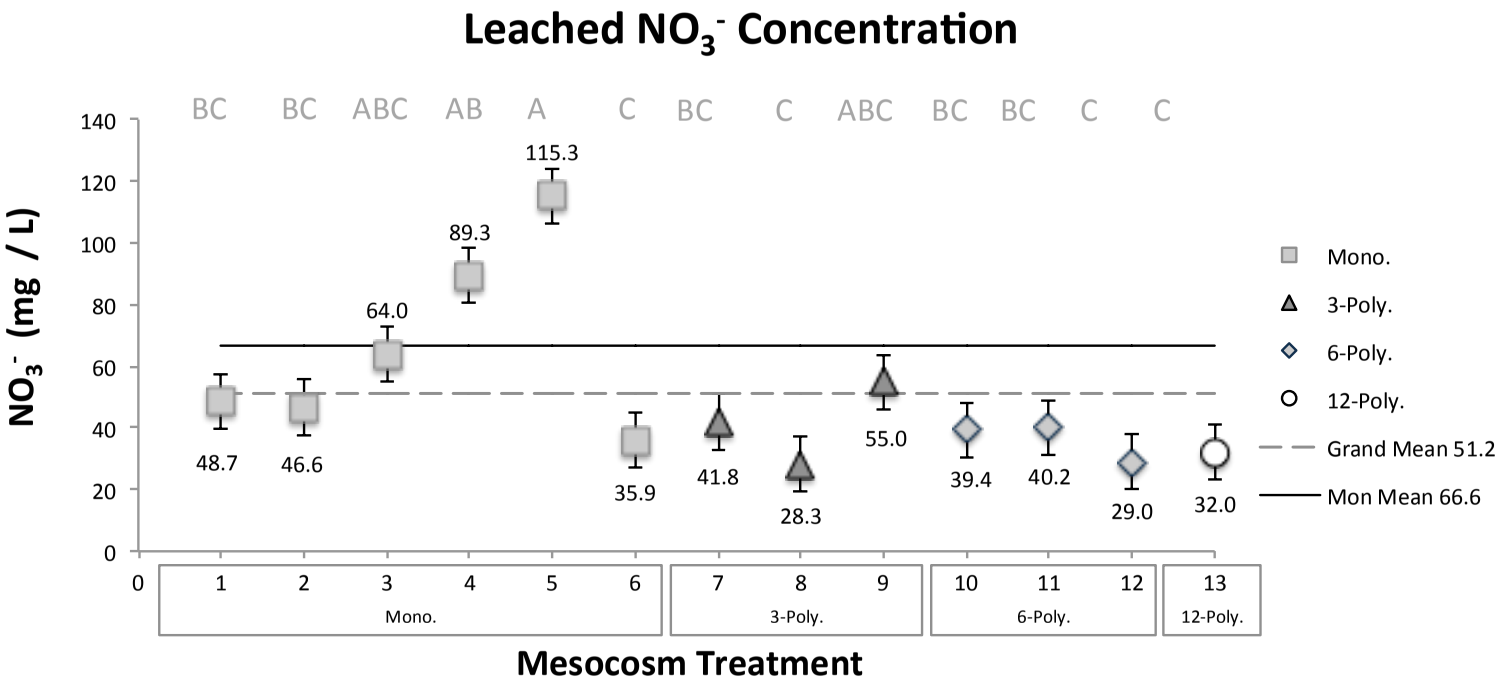

Figure A. 5 – Leached NO_3^- concentrations by treatment suggest species effects among monoculture treatments, but less clearly than in productivity measures. Treatment 5 (*Trifolium repens*), a legume, leachate contained the highest concentration of NO_3^-, however, was not significantly different from Treatment 3 or 4 (*Festuca rubra* 'Garnet' and *Poa pratensis* 'Bedazzled', respectively). Treatment 6 (*Festuca arundinacea* 'Falcon V') has the lowest NO_3^- concentration of the monocultures. Grey letters indicate differences via a Tukey test. 3- and 6-component polycultures containing *T. repens*, leached NO_3^- at higher concentrations than polycultures without *T. repens*, but were not significantly different. Treatments containing Error bars represent ±1 SEM. Bars with contrasting letters indicate significantly different means. Significance testing was performed utilizing a square root transformation.